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S. P. WILTSHIRE.

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THE INTERNAL TEMPERATURES OF FRUIT-TREE BUDS

BY JOHN GRAINGER, PH.D. AND A. L. ALLEN, PH.D.

(With 10 Text-figures)

FROST takes a severe toll from the harvest of fruit in every year. The damage done is not merely an effect of the freezing of water or of a dilute solution. Church and Ferguson (2) have found that the air temperature must fall to 27° F. to kill apple buds, but only to 29° F. to kill the flowers, and to 30° F. when the fruit is setting. Johnston (4) also found a similar phenomenon, and termed it "undercooling". The Duke of Bedford and Spencer Pickering (1) distinguished between radiation frosts and wind frosts, and mentioned the fact that heat may be lost from the bud by radiation to the surrounding air at night. Bud scales have been supposed to serve as insulation to conserve the heat generated by the metabolism of the tissues within, and this hypothesis has often been used to explain the foregoing observations on damage by frost.

A simple class experiment showed that considerable amounts of water were lost through dormant buds. Three sets of similar apple twigs were weighed. One set was vaselined all over, another had only the buds covered with vaseline, and the third was left untreated. All were reweighed after a week's exposure to the air. The untreated twigs lost 28 per cent. of their weight, whilst the other two sets each lost about 11 per cent. These results show that water vapour is lost from a twig mainly through the buds.

Miller and Saunders (5) and Seybold (6), using thermoelectric methods of estimating temperatures, showed that leaves were often cooled below the temperature of the air by transpiration. If transpiration from dormant buds has the same effect, one would expect that on frosty nights the bud would reach freezing-point before the surrounding air. The observed facts quoted above show that buds are not damaged by frost until the air temperature falls well below 32° F. There is considerable circumstantial evidence that a dormant bud possesses some compensating mechanism against damage by frost.

The present investigation was designed to study the relation between the temperature of the air and that within a bud at any time during the day or night, and under varying climatic conditions. It was also hoped that the records would include a few frosts, and although these were not numerous, they yielded some interesting data.

EXPERIMENTAL METHODS

Thermoelectric circuits were used to measure the temperatures of buds, and of the surrounding air. Thermocouples were made in three sections. The actual junction consisted of two fine wires (40 S.W.G.) of iron and constantan soldered together. They were insulated at all places except the junction by a coat of cellulose varnish. Wires of progressively larger gauge were attached (Fig. 1), and the connections to the measuring galvanometer were made with large gauge double cotton-covered copper wire.

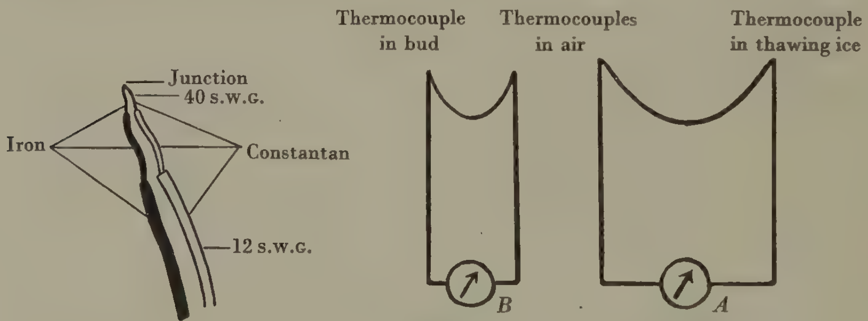


Diagram of thermocouple

Temperature difference between bud and air

Temperature of the air

Fig. 1.

Two Cambridge recording galvanometers of the frictionless, inked thread pattern were used for measuring the currents. They gave a movement on the scale of approximately 3 mm. per ° C. Records of dots made every 2 min. were printed upon paper charts stretched upon a cylindrical drum revolving once in 24 hours.

Buds of apple (Bramley's Seedling and Worcester Pearmain), black currant (Seabrook's Black) and raspberry (Lloyd George) were used for experiment. A hole was pricked into the bud by means of a fine needle. The thermocouple was pushed in gently, and the larger wires were tied to the branch bearing the bud, so that even a high wind did not disturb the arrangement. Wounds caused by the needle pricks produced no initial rise in temperature, though a small amount of callus was formed later. They did not affect the records in any instance.

Records were taken continuously from mid-March to mid-May in 1932 at the Leeds University Fruit Station at Osgodby, near Selby, and from April 1 to the middle of May 1933 at the first-mentioned writer's garden in Adel, Leeds. Experiments at Osgodby were confined to a study of apple buds; those at Adel dealt with apple, black currant and raspberry.

Measurement of air temperature

Each galvanometer was arranged to measure the thermoelectric current due to the difference in temperature between two thermocouples; if one couple were placed in thawing ice (*i.e.* at 0° C.) and the other were in the air, the galvanometer would record fluctuations from which the actual temperature of the air at any moment could be calculated directly. Such a record was obtained upon galvanometer *A* (Fig. 1). The thermojunction in the air was covered by a conical screen of zinc, which protected it from rain and mechanical damage, but no considerable difference in temperature was ever observed between a screened and an unscreened thermocouple, except during rain. Zero points on the curve, indicating the position of 0° C., were obtained by disconnecting the circuit for 10 min. each day.

Measurement of bud temperature

The thermoelectric current representing the difference in temperature between a thermojunction in a bud and another junction in the air, was recorded upon galvanometer *B* (Fig. 1). The junction in the air was placed under the same screen as the air thermocouple mentioned in the last paragraph, and the actual bud temperatures could be obtained by adding the curves of galvanometers *A* and *B* algebraically. Zero points on the records, representing no difference between the temperatures of the air and of the bud, were obtained by disconnecting the circuit as before.

Simultaneous records of circuits comparing the temperatures of two buds were occasionally made upon one galvanometer drum. A two-way switch, operated by the clockwork recording mechanism of the galvanometer, switched over to each of the two circuits alternately. The two curves were separated on the chart by incorporating a small current, provided from an accumulator, and standardised by a potentiometer, in one of them (Fig. 2). When the second pair of thermocouples was in circuit, the pointer of galvanometer *B* would therefore swing a certain standard distance, plus or minus the current produced by the difference in temperature. Zero points were obtained by disconnecting the thermocouples as before, but a resistance, equal to that of the outdoor part of the circuit, was required in the circuit with the added current (Fig. 2).

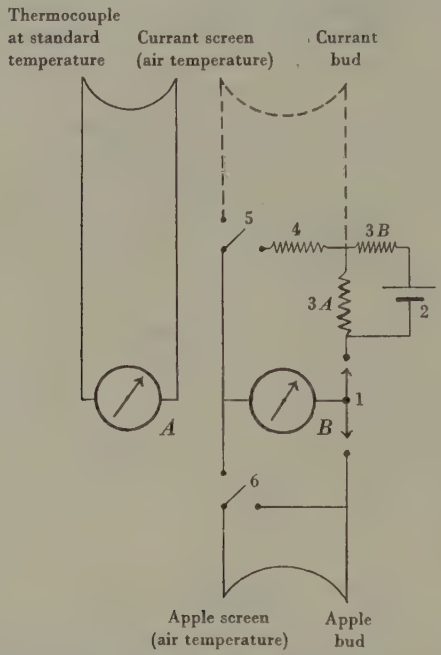

Thermocouple at standard temperature
Currant screen (air temperature)
Currant bud
5
4
3B
3A
2
1
A
B
6
Apple screen (air temperature)
Apple bud

Fig. 2. Arrangements for the simultaneous production of two records upon one recording galvanometer (*B*). 1, two-way switch operated by the recording mechanism of the galvanometer. 2, accumulator. 3*A*, resistance of 0·01 ohm. 3*B*, resistance of 200 ohms. 4, resistance equal to the out-of-doors part of the circuit (*i.e.* the part shown by a dotted line in the diagram). 5 and 6, zero switches.

RESULTS

Apple

Seventy-eight out of the eighty-two daily records of the temperature of a dormant or slowly opening Bramley's Seedling apple bud made in 1932 and 1933 show that the bud was warmer than the air during the day, and cooler during the night (Figs. 3 and 4). Only four instances were recorded when the bud was warmer than the air during the night as well as the day. The difference was not more than 1° C. in any of the four instances.

No varietal difference in temperature was observed between Worcester Pearmain and Bramley's Seedling buds, for records made continuously for 22 days in 1932 showed a very close coincidence, both as regards amount and direction of the variations (Fig. 5).

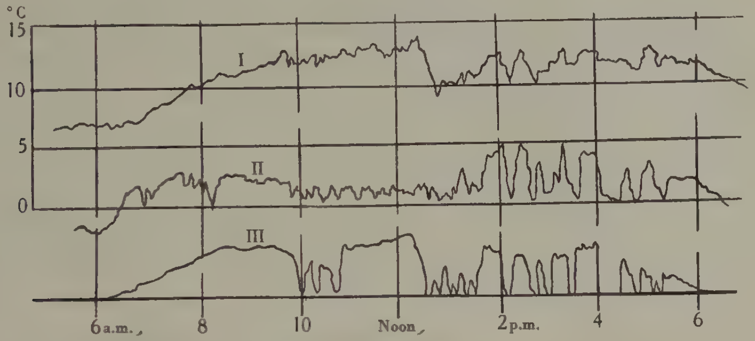


Fig. 3. I. Air temperature. II. Temperature difference: temperature of apple bud *minus* temperature of air. III. Intensity of sunshine (estimated by examination of sunshine card).

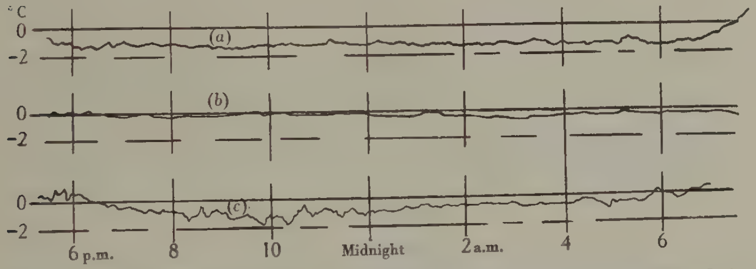
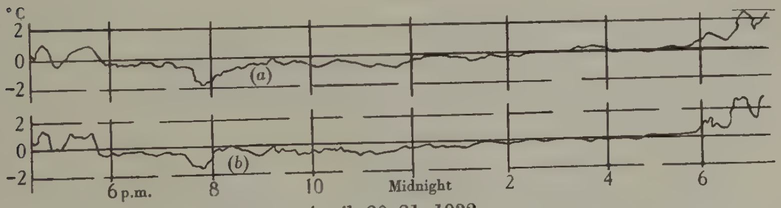


Fig. 4. Temperature difference: temperature of bud *minus* temperature of air. (*a*) March 24–25, 1932. (*b*) May 1–2, 1932. (*c*) May 10–11, 1932.

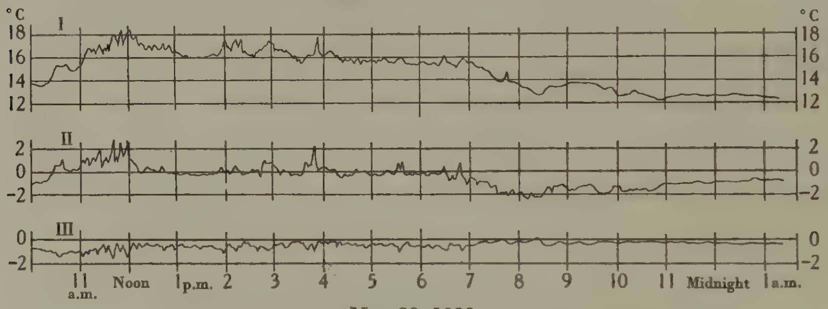


April, 20–21, 1932

Fig. 5. Temperature difference: temperature of bud *minus* temperature of air. (*a*) Bramley's Seedling. (*b*) Worcester Pearmain.

Cooling of the bud during the night would seem to be mainly a direct result of loss of heat by evaporation. Loss of heat by radiation from the bud to a clear sky at night is probably an important secondary factor. The zinc screen placed over a bud would eliminate loss of heat by radiation, and screened buds were rarely as cool as unscreened buds during the hours of darkness (compare II and III, Fig. 6).

The rise in temperature during the day can be explained by assuming that the bud receives radiant heat from the sun. A sharp rise in the temperature of the air corresponds to a rise in temperature of the bud above that of the air. The bud, being a solid object, is warmed more than the air. This point was tested by comparing the temperature of an unscreened bud with that of a bud shielded from the sun's radiant heat under a conical screen of zinc. The results are shown in Fig. 6, which is typical of twenty daily records. Most illuminating results come from the records in daylight. The unscreened bud was, as usual, warmer than the air; its peaks corresponded directly with the peaks of the air-temperature curve (Fig. 6). The temperature of the screened bud was lower than that of the air even during the day, and a coincidence of the peaks of the air-temperature curve with the valleys of the screened-bud curve was seen. A rise in air temperature caused a lowering of the temperature of the screened bud. Raising the air temperature decreases its relative humidity; evaporation from any source of water vapour is thereby increased, and more latent heat of evaporation is taken from the bud, thus lowering its temperature.



May 22, 1932

Fig. 6. I. Air temperature. II. Unscreened bud. III. Screened bud. II and III show temperature difference, *i.e.* temperature of the bud *minus* temperature of the air.

These results show that the warming during the day is by direct radiation from the sun, and confirm the view that lowering of the temperature of a bud can be caused by evaporation. The amount of fall in temperature at night was usually less than that of the screened bud during the day. Evaporation would usually be greater during the day, but would nevertheless take place steadily at night.

Black currant

Flower-containing buds of black currant were used for experiment in 1933. Seventeen daily records were obtained between April 14 and May 2. The temperature of an apple bud was recorded upon the same chart in each case. All the records showed the rather surprising fact that black currant buds were usually cooler than the surrounding air during the daytime. Their temperature curves were, in fact, roughly the inverses of those portraying the state of the apple buds (Fig. 7). An explanation of this phenomenon is suggested by the fact that the black currant buds were further open than the apple buds in the latter half of April; they were therefore more susceptible to the cooling effects of evaporation, which would, as shown above, increase as the temperature rose, if heating by radiation did not counterbalance it.

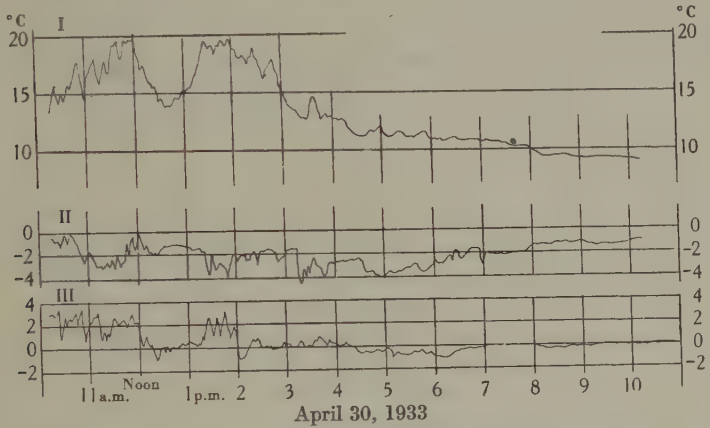


Fig. 7. I. Air temperature. II. Unscreened black currant bud; temperature of bud *minus* temperature of air. III. Unscreened apple bud; temperature of bud *minus* temperature of air.

Raspberry

Four records, made between May 2 and 5, 1933, showed that flower buds of raspberry usually exhibited similar variations to those of black currant buds, except that fluctuations in the former were usually not so large as in the latter. Raspberry buds were, however, warmed considerably during bright sunlight. They may, so far as these few records show, be regarded as intermediate between black currant and apple in the matter of their temperature relations.

Frost

A frost occurred on the morning of May 8, 1932, whilst these records were being taken. Minima of 28·5° F. in the screen and 25·5° F. on the ground, were recorded at the Osgodby station. This frost caused severe reduction in subsequent yield of gooseberries and currants both red and black, but did not damage apples or plums.

The galvanometer record (Fig. 8) showed that the more severe part of the frost lasted for only an hour and a half, and that when the temperature of the air fell below freezing-point, the temperature of the bud was higher than that of the air. This was unusual during the hours of darkness, and may, perhaps, be due to the liberation of latent heat of fusion of the plant juices if parts of the bud had actually begun to freeze. Freezing of the outer scales would stop evaporation, and the latent heat of fusion would raise the temperature within. This explanation would only account for the maintenance of a higher temperature in the bud for a certain time—until freezing was complete—and it may be that the buds were saved from being killed by the fact that the more severe part of the frost was of short duration.

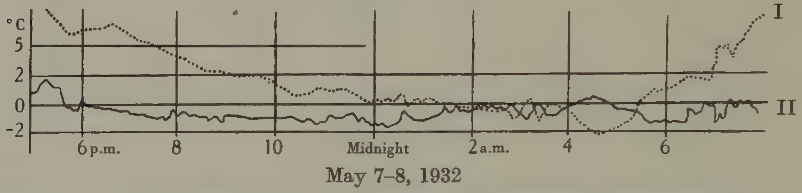
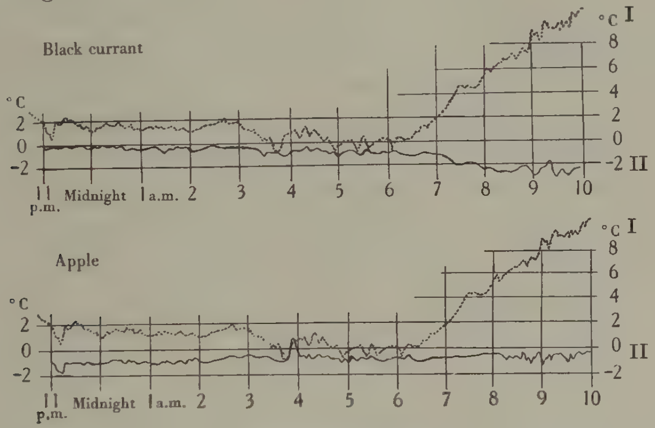


Fig. 8. I. Air temperature...... II. Temperature difference: temperature of bud *minus* temperature of air———.

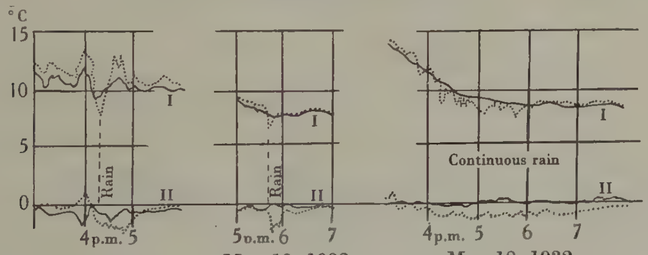
A slight frost occurred on April 14, 1933. The air temperature fluctuated round about 32° F. for over 2 hours. This was not sufficient to damage either the apple or the black currant buds, but the results help to explain the observations of the previous year, when black currants were damaged, but not apples. The curves (Fig. 9) show that the bud temperature of the black currant was uniformly below the air temperature, and a depression in the latter was followed, after a short time lag, by a depression in the former. The temperature of the apple bud rose above that of the air for the brief period, shortly before 4 a.m., when the air temperature was about 30° F. for a quarter of an hour. Although the frost was not severe, the records were very definite, and would seem to confirm the idea that the apple bud has a mechanism for frost resistance not possessed, or at least not shown in the same degree, by the black currant.

Black currant buds were bursting on April 13, 1933, whilst apple buds were still dormant. If the explanation that the outer bud scales actually begin to freeze, and liberate latent heat of fusion, is correct for apple, it would also illuminate the behaviour of black currant. The young expanding leaves and flowers of this plant are the parts which freeze—they have no bud scales at this time. Hildreth (3) also found that the killing temperature of buds rose quickly in spring, when they were opening.



April 13–14, 1933

Fig. 9. I. Air temperature....... II. Temperature difference: temperature of bud *minus* temperature of air———.



May 25, 1932 May 19, 1932 May 18, 1932

Fig. 10. I. Air temperature: screened thermocouple———, unscreened.......
II. Temperature difference: screened bud———, unscreened.......

Rain

Rain, falling directly upon a thermojunction or a bud, causes a sudden fall in temperature, followed by a rise after a considerable time (Fig. 10). Screened thermocouples or buds do not show any fall. Lowering of the temperature is evidently brought about by evaporation of the rain.

SUMMARY AND CONCLUSIONS

1. The temperatures of dormant and opening fruit buds of apple, black currant and raspberry have been investigated by thermoelectric methods.

2. Apple buds are warmer than the air by day, and usually cooler at night. Warming seems to be effected by radiation from the sun, since a screened bud was cooler than the air during the daytime as well as at night. Cooling would seem to be a direct result of evaporation from the bud, with radiation as an important secondary factor on clear nights.

3. Black currant and raspberry buds are usually cooler than the air at all times. Buds of these plants were opening, whilst apple buds were dormant, and it would seem that loss of heat by evaporation dominates even the warming effect of the sun during the day.

4. Apple buds seem to have a mechanism of resistance against frost, whilst no evidence of such protection has been found in black currant buds.

5. Results are discussed further under the various headings of the text.

In conclusion, we desire to express our sincere thanks to Prof. J. H. Priestley for extensive help in the prosecution of the investigation here recorded, to Mr J. W. Eves of Leeds University, and to Mr A. Potts of the Fruit Station, Osgodby, for valuable assistance in obtaining the 1932 records.

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PHYTOPHTHORA INFESTANS (MONT.) DEBARY AND *CLADOSPORIUM FULVUM* COOKE ON VARIETIES OF TOMATO AND POTATO AND ON GRAFTED SOLANACEOUS PLANTS

By T. E. T. BOND, M.Sc.

(*From the Department of Agricultural Botany, University of Reading*)

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I. Introduction

The use of grafting as an experimental method has become increasingly prominent in plant disease investigations and in the study of certain aspects of physiology. In practical horticulture, grafting has been used from time immemorial for the propagation of deciduous and evergreen fruits and various ornamental trees and shrubs. A large body of empirical information connects the use of certain stocks with the production of certain desired characteristics, generally of a purely horticultural nature affecting vegetative vigour and longevity or frequency and relative abundance of cropping. In many cases, the possibility of altered disease relationships has been entirely overlooked. On the other hand, several stocks formerly in use have been discarded owing to the prevalence of scorch and other nutritional disorders induced in the trees grafted upon them. The various practical and theoretical issues raised by the disease relationships of grafted plants have been fully discussed in a previous paper (9). The purpose of the present communication is confined to a single issue only, namely, the possibility of an induced or transmitted increase or decrease in susceptibility to invasion by pathogenic fungi.

The results reported by previous workers have often been entirely negative, both stock and scion retaining their characteristic reaction to infection unchanged. The best-known examples are those of Fischer (15) and Sahli (36), on the effect of grafting on the susceptibility of various Pomaceae to infection by *Gymnosporangium* spp., and of Roach (34, 35) and Köhler (21), on varietal immunity of potatoes to wart disease, caused by *Synchytrium endobioticum* (Schilb.) Perc. The absence of any effect of grafting on disease relationships of stock and scion was also reported by Gibson (16) for chrysanthemum rust, *Puccinia chrysanthemi* Roze, by Salmon and Ware (38) for the powdery mildew of hops, *Sphaerotheca humuli* (DC.) Burr., by May (26) for tomato wilt, *Fusarium lycopersici* Sacc., and by Leach (23) for bean anthracnose, *Colletotrichum lindemuthianum* (Sacc. and Magn.) Bri. and Cav. Negative results, while necessarily inconclusive, indicate "...that, if resistance or susceptibility is due to a specific substance in the plant, this substance is not transmitted from stock to scion or *vice versa*, or else it is modified by the cells which receive it" (23). Positive results were reported by Klebahn (20) for the uredial stage of the white pine blister rust, *Cronartium ribicola* Wald., and by Blaringhem (8) for hollyhock rust, *Puccinia malvacearum* Mont. In both cases, scions which were normally resistant were found to be capable of infection when grafted on a susceptible stock. There is no evidence, however, to show that a similar increase in susceptibility could not have been induced by other means. The early experimental investigations were concerned almost entirely with the possibility of direct transmission of resistance or susceptibility; recent observations emphasise the possibility of an indirect effect resulting from a modification in the normal response to environmental conditions. This outlook is exemplified by the work of Volk (43), in 1931, in which experiments with grafted plants form part of an extensive series of observations on the relation of environmental factors to the development of disease. The grafting experiments were confined to potato blight, caused by *Phytophthora infestans* (Mont.) de By., and tomato leaf mould, caused by *Cladosporium fulvum* Cke. Special attention was paid to the state of vigour of the host plant, as influenced by grafting, which, under certain conditions, might have an adverse effect on the development of the parasite. In no case, however, was immunity to disease affected. In several respects, Volk's account of his experiments is by no means satisfactory, and his conclusions will be considered later, in connection with the present work.

In so far as the choice of material is the same, the present work may be regarded as a confirmation of Volk's experiments. However, their scope has been extended to include observations on varietal relationships as well as on the purely specific host range of the two fungi. The use of varietal relationships permits the reciprocal influence of stock and scion to be studied more fully. If grafting induces changes in one component in any way related to the reaction of the other, there will be better justification for the assumption that direct transmission of the factors responsible has occurred. Otherwise, the effect must be indirect, and will be indistinguishable from a modification induced by changed environmental conditions. Negative results cannot be conclusive in themselves, but the suggestions that they afford may be further supported or confirmed by circumstantial evidence derived from the preliminary experiments and considerations in each case.

II. MATERIALS AND METHODS

(1) *Host plants*

Potato.

Nine varieties were available, as follows: 1*st Earlies*—Arran Crest, Duke of York; 2*nd Earlies*—Arran Comrade, Stirling Castle; *Early Maincrop*—The Ally, Great Scot, King Edward; *Maincrop*—Golden Wonder, Up-to-Date. Both "immune" and "non-immune" varieties were represented in each class. Grade I Scotch seed was used throughout. The tubers were sprouted in pots in a glasshouse and the first series were planted in pots in March. The remaining series were planted out in the usual way from the middle of April, using a piece of newly broken grassland, without addition of artificial fertilisers. By planting in lots, at intervals, they were obtained in varying stages of development throughout the summer. All varieties remained fairly free from virus diseases.

Tomato.

A selection was made from seedsmen's lists, to include as many different forms as possible. The following varieties were grown, namely: red fruited—Giant Red, Kondine Red, Plentiful, Up-to-Date, Maincrop, Stirling Castle, Superlative, Earliest-of-All, Open Air; yellow fruited—Golden Queen, Large Yellow. In addition, a number of small-fruited forms were grown from a mixed packet of seed supplied by Messrs

Suttons. Three types were obtained, which are described below as S.F. 1, S.F. 2, and S.F. 3 respectively.

S.F. 1. Of more slender habit, but otherwise similar in vegetative characters to the ordinary tomato, *Lycopersicum esculentum* Mill. Includes "cherry", "plum" and "pear" type fruits, representing vars. *cerasiforme* and *pyriforme* of Bailey (4) and other writers.

S.F. 2. Has the characteristic foliage of var. *grandifolium* Bailey (4), with large, entire terminal leaflets. The fruit is intermediate in shape between "pear" and "plum".

S.F. 3. The "currant" tomato. This is a very distinct plant, of slender, branching habit, and with smaller, entirely glabrous leaves. The leaflets are rounded and often almost entire, and the secondary leaflets are very small. The fruits are of the size of currants, approximately 0·7–1·3 cm. in diameter, and are borne in long two-rowed racemes. The true identity of this form remains uncertain. The currant tomato has been variously described as *Lycopersicum pimpinellifolium* Dun. and as *L. racemigerum* Lange. Bailey (4), with whose description the plant is in entire agreement, considers the two species to be identical, giving as additional synonyms *L. racemiforme* Lange and *Solanum racemiflorum* Vilm. The S.F. 2 type was found to agree closely with plants grown from seed supplied from Kew as *Lycopersicum racemigerum*. On the other hand, the type in cultivation at the John Innes Horticultural Institution, supplied through the kindness of Dr F. W. Sansome, differed in several important respects. The plant was hairy, and in habit and in the shape of its leaves bore a greater resemblance to *L. esculentum*. The results of inoculation experiments show that all three forms are immune to *Cladosporium fulvum*.

All tomatoes were grown from seed in a mixture of old garden loam and leaf mould without artificial fertilisers.

Other Solanaceae.

Solanum nigrum, *S. dulcamara*, *Atropa belladonna*, *Datura stramonium*, *Physalis* sp. and *Hyoscyamus niger*. With the exception of the first two, which were grown from seed collected in the neighbourhood, these were supplied as cuttings from the University Agricultural Botanic Garden.

(2) *Culture of* Phytophthora infestans

Source of material. Diseased material was collected personally and also obtained from the following workers to whom the writer is indebted: A. Beaumont, A. Dillon Weston, Dr G. H. Pethybridge, Dr T. Small, and Dr W. M. Ware.

Isolation of pure cultures. The diseased tubers were surface-sterilised and then cut in half and left in a moist chamber at an average temperature of about 10° C. Under these conditions, aerial mycelium developed from the cut surface in 2–3 days. The tubers could later be resterilised and cut so as to encourage the development of fresh mycelium. In many cases, however, the presence of other tuber rotting fungi, principally *Fusarium* spp., made a transfer to fresh tubers desirable. The removal of bacterial contamination was effected by growing on aseptic potato slices in Petri dishes, as recommended by Crosier (11) in 1933. The slice cultures were held at 10° C., the humidity being kept always slightly below 100 per cent. Under these conditions, aerial mycelium was encouraged to develop beyond the region of contamination, but if the humidity was allowed to decrease too far, the mycelium soon dried out and was useless for subculturing. A return to 100 per cent. humidity was sometimes effective, but was liable to result in the sudden spread of the contamination over the entire surface of the slice. Successful isolations to artificial media were obtained from slice cultures, and, in a few cases, from diseased tubers. The slants were maintained at 100 per cent. humidity, generally in "Kilner" jars with loosely fitting lids, and the plugs were covered with "Cellophane". A portion of aerial mycelium was used as inoculum, except where bacterial contamination was suspected. In the latter case, the surface of the culture was touched lightly with a platinum loop containing sterile water, so that conidia only were removed. In making transfers from existing slant cultures, the inoculum consisted of a portion of medium containing submerged mycelium. The best way of increasing the number of cultures was to transfer to potato slices, and inoculate the new slants from these.

A number of different agar media were tried, of which the most suitable was found to be "Bacto" Lima bean agar. Even on this medium, growth was slow, and plate cultures failed completely owing to the more rapid drying out of the medium. Placing the remaining media roughly in order of suitability, judged both by the extent of growth and by the amount of aerial development, yellow corn and potato-dextrose-peptone were best, then oatmeal, "Bacto" corn meal and synthetic solution, and, finally, tomato fruit agar. The media tried included the recommendations of Clinton (10), Jones *et al.* (19), Pethybridge and Murphy (31), and others, but in no case was growth so extensive as reported by these authors. On Lima bean agar, which was used exclusively for the maintenance of stock cultures, a dense submerged growth was produced, in which several distinct types of structure were identified. These included a single parthenogenetic oogonium, agreeing closely with the description given by Pethybridge and Murphy (31) in 1913.

Germination of conidia. Conidia were removed from the majority of the slice cultures and their germination observed at laboratory temperature (13–15° C.) and also in an incubator at 25° C., in both cases in hanging-drop cultures. The method of germination was found to depend on the temperature and on the age of the cultures (which were maintained at laboratory temperature). At 13–15° C. direct germination was observed after 11 days, and indirect germination after 6 days: at 25° C. direct germination occurred after 7 days, and indirect germination only after 8–12 days. In both cases, the percentage of direct germination was low. Direct germination resulted in the production either of a single germ tube or of "secondary" conidia, as described by Crosier (12). Indirect germination was observed on several occasions, from nine to fifteen zoospores being liberated from a single sporangium. At the lower temperature, the whole process was very rapid, most of the conidia germinating within 2 hours. Germination of the zoospores proceeded with equal rapidity. After a short period of motility, the spores came to rest and developed a cell wall, germinating immediately by putting out a single germ tube. This remained unbranched for 24 hours, becoming separated from the empty spore by a single cross-wall near the base.

(3) *Culture of* Cladosporium fulvum

Single spore cultures were isolated from diseased tomato foliage obtained from various local nurseries, and oatmeal agar was the most satisfactory medium. The colonies produced free spores for as long as 3–4 months, increasing slowly in size without any appreciable change in appearance. The optimum temperature for growth appeared to be about 25° C. The colour[1] varied from medal bronze (19 YO–Y.m.) to deep olive buff (21‴ O–YY.b.), and the colonies differed from those of *C. herbarum* on the same medium only in colour and in their slower rate of growth. On Czapek's and other media, however, growth was characterised by the early cessation of sporulation and the development of the stromatic condition. After 4–5 weeks at 13–15° C., the colonies consisted of an outer, dense, stromatic shell covering an inner mass of disorganised hyphae and bearing a white felt of sterile mycelium on the outer surface. The mycelium developing from freshly exposed parts of the colony was also entirely sterile.

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[1] See Ridgway's *Colour Standards and Colour Nomenclature.*

Morphology. Viable conidia were obtained from cultures on oatmeal agar at all ages, from 4–12-day-old cultures on Czapek's agar, and also from infected foliage. Leaf conidia were germinated in standard foliage decoctions from the following plants, namely, Giant Red tomato, small-fruited tomatoes types 2 and 3, *Lycopersicum racemigerum* (Kew), and King Edward potato (2 per cent. decoction for 3 min. in boiling water). In each case, distilled water was used as a control. Germination in decoctions from the (immune) type S.F. 3 and *L. racemigerum* was the same as in distilled water, showing that immunity could not depend on the presence of a water-soluble substance capable of inhibiting spore germination in standard decoction, as assumed by Schmidt (39). In Giant Red tomato and type S.F. 2, the germ tubes were slightly more branched. Germ tubes of the knotted or "BB" type of Schmidt (39) were observed only in the decoction of King Edward potato, although even in this case in presence of a number of intermediate types as well. According to this author, the production of the "BB" type of germ tube in decoctions of potato and tomato depended on the presence of small quantities of solanin. However, in a later paper, Agerberg *et al.* (2) pointed out that Schmidt had carried out his experiments entirely with conidia obtained from artificial culture. With leaf conidia, on the other hand, the effect of solanin was much less pronounced; germination in decoction of *L. racemigerum* was also normal. These observations were confirmed in the present investigation. The production of the "BB" type of germ tube by culture conidia has been developed as an extremely delicate test for the detection and estimation of small quantities of solanin (1).

Mature conidiophores from a culture on tomato decoction showed sympodial development with the production of 1–2-celled spores in chains, typical characters which are described in Rabenhorst's (32) *Kryptogamen-Flora*, and were confirmed by Makemson (25) in 1918. On the other hand, the illustration of this species given by Schmidt (39) resembles a totally different organism—*Trichothecium roseum* Lk. The habit of growth on artificial media described by Schmidt also suggests this species rather than *Cladosporium fulvum*. So far there has been no evidence for the existence of biological strains of the latter organism, differing in host relationships, or widely separated by cultural characteristics. Added to these considerations, the fact that the results of Agerberg *et al.* (2), using leaf conidia (which could hardly have been other than *C. fulvum*), have been confirmed, raises the possibility that, in artificial culture, Schmidt's *C. fulvum* was contaminated by *Trichothecium* *roseum* or a similar species. A repetition of Schmidt's observations is desirable.[1]

(4) *Technique for inoculation experiments*

Detached leaves in Petri dishes were used for the preliminary inoculations and then, where necessary, the results were confirmed by inoculating seedling and older plants in pots. In all cases uninoculated controls were maintained. In each experiment, a minimum of four leaves was used for each variety. The leaves were washed in tap water and rinsed in sterile water before placing in Petri dishes. The dishes contained moistened filter paper and were stacked so as to receive as much diffuse light as possible. No mineral salts were supplied, the filter paper being moistened when necessary by addition of distilled water only. Leaves kept under these conditions sometimes remained green for as long as 5 weeks, often putting out adventitious roots from the base of the petiole. No difficulty was experienced with leaves from plants grown under glass, but where the plants had been grown out of doors, there seemed to be greater danger of "breakdown" and bacterial contamination. Actually, the danger of contamination in the *Phytophthora infestans* inoculations was not great, since the experiments were rarely more than 10 days in duration. With *Cladosporium fulvum*, on the other hand, the experiments often lasted for as long as 3–4 weeks, and here the use of outdoor-grown potatoes and tomatoes was almost entirely prevented. Less difficulty was experienced with *Solanum nigrum*, *S. dulcamara*, and the other Solanaceae. Seedling and grafted plants were maintained in a propagating frame inside a glasshouse to ensure a high relative humidity.

Both conidia and aerial mycelium were used as the source of inoculum for *Phytophthora infestans*. The conidia were removed from 6–9-day-old slice cultures and were allowed to germinate at 13–15° C. The suspension of zoospores was afterwards applied by means of a de Vilbiss atomiser. Spore suspensions of *Cladosporium fulvum* were obtained from colonies on Czapek's medium, not more than 10 days old, or from material infected in one of the previous experiments. No loss of virulence was experienced after prolonged periods of growth in artificial culture.

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[1] Since this communication went to press, a paper by Raabe and Sengsbusch has been received (*Gartenbauwiss.* (1935), IX, 183–8), in which it is stated that Schmidt and his collaborators confused *Cladosporium fulvum* with *Trichothecium roseum*.

(5) *Technique for grafting experiments*

In all some fifty grafts were made, involving various combinations of tomato (both commercial and S.F. types), potato, *Solanum nigrum*, *Atropa belladonna*, and *Datura stramonium*. The tomatoes and some other plants were in 6-in. pots, and were 5–8 weeks old. Detached shoots of potato and *Atropa belladonna* were obtained from plants growing in beds. All but the very young side shoots were hollow and unsuitable for grafting. In general, the condition of the material could be judged best from the appearance and "feel" of the stem when cut through with a razor. When both cut ends remained firm, but with abundant exudation of sap, successful "take" of the graft could be predicted with considerable certainty. The simple cleft graft was found to be the most convenient. Stock and scion were chosen so that their cut ends were of the same diameter and, usually, the apical portion of the scion was removed so that only the two lower leaves remained. The graft was tied with moistened raffia. The grafted plants were maintained in a propagating frame at 100 per cent. humidity for at least 2 days, and were then given increasing ventilation until their removal to the glasshouse bench at the end of the second week. As the plants increased in size, they were staked, and the raffia was cut to prevent constriction of the growing stem.

III. EXPERIMENTAL RESULTS

(1) *Varietal relationships*

(*a*) *Phytophthora infestans.*

Potato. The general course of events was in agreement with the majority of the published accounts, three distinct stages of infection being distinguishable, as follows: (*a*) a pale or greyish discoloration, (*b*) development of aerial mycelium, (*c*) blackening and "water-soaking". The progress of the disease was recorded throughout the inoculation experiments for each variety, but no constant differences were observed either in incubation period or in intensity of infection. In all cases, the upper surface was slightly more resistant than the lower. An attempt was also made to distinguish between the varieties by measuring the rate of spread of infection by the method used by Jones *et al.* (19) in 1912, but it was soon realised that this would involve a statistical analysis beyond the scope of the present investigation. Differences in the inherent susceptibility of different varieties undoubtedly occur (33, 44), but they are slight, and are frequently masked by the effects of age and period of maturity (12), habit (37), climatic conditions (14), and other factors influencing what Beaumont (5) has recently described as the "epidemic potentiality" of the crop as a whole.

Table I

Inoculation experiments with Phytophthora infestans.
Tomato and other plants

Name of plant	No. of exps.	Results			
		Definite	Slight	Doubtful	Negative
Tomato:					
Giant Red	5	////	—	/	—
Golden Queen	5	///	/	/	—
Stirling Castle	5	—	/	/	///
Large Yellow	4	/	—	/	//
Up-to-Date	4	—	/	/	//
Plentiful	4	—	—	//	//
Earliest of All	4	—	—	/	///
Kondine Red	4	—	—	/	///
Superlative	4	—	—	/	///
Open Air	4	—	—	/	///
Maincrop	5	—	—	—	/////
S.F. 1	5	—	/	///	/
S.F. 2	2	—	—	//	—
S.F. 3	5	///	/	—	/
Other plants:					
Solanum dulcamara	3	—	—	—	///
S. nigrum	2	—	/	/	—
Atropa belladonna	3	—	—	/	//
Hyoscyamus niger	2	—	—	/	/
Datura stramonium	2	—	—	/	/
Physalis sp.	2	—	—	—	//

Each mark "/" represents a record in a single experiment.

Tomato and other plants. The results with tomato and other hosts are presented in Table I. Three degrees of infection are recognised, namely, "definite", "slight", and "doubtful". "Definite" infection, for instance, on Giant Red tomato, is characterised by the appearance of sharply defined blackened areas having comparatively scanty aerial mycelium, and without any tendency towards the rapid spread of "water-soaking" as in the potato. Infection was judged to be "slight" when the lesions were limited in number or when no aerial mycelium was produced until after the seventh day from inoculation. After local inoculation, the infected area remained small, with little fresh aerial mycelium. "Doubtful" infection represents any symptoms not observable in the controls and resembling incipient stages of infection, but in no case with any development of aerial mycelium. Table I shows that Giant Red and Golden Queen are clearly the most susceptible of the eleven tomato varieties, while Maincrop is the most resistant, being the only variety for which the results were uniformly negative. Of the small-fruited tomatoes, the currant or S.F. 3 type only is susceptible. Except for a single record of "slight" infection for *Solanum nigrum*, the results for the other Solanaceous plants investigated are "doubtful" or negative. The symptoms of "definite" infection on the tomato agree with those recorded in similar experiments by Melhus (27) in 1916 and by Giddings and Berg (17) in 1919. On the other hand, the fact that certain varieties remained immune suggests a possible explanation of the negative results obtained by Smith (42), Wiltshire (45), and Reddick ((33), p. 499). Naturally infected tomato foliage was received from Jersey, C.I. The symptoms were those of typical potato blight and would therefore appear to be due to infection by the "tomato" strain, as described by Berg (6) in 1926. However, there has been no opportunity of performing inoculation experiments with this strain, although cultural observations indicate that it is less readily grown on Lima bean agar and other media than the "potato" strains. The present results are in no way contrary to Berg's conclusions, and demonstrate in addition the existence of well-marked differences in the susceptibility of tomato varieties to the "potato" strains.

(*b*) *Cladosporium fulvum*.

On all tomato varieties, the early stages of infection are characterised by the appearance of an extremely diffuse, grey, aerial mycelium, without any discoloration of the tissues. As sporulation commences, the fungus assumes a brownish coloration, which later becomes dark, tawny, or occasionally purplish. Arranged according to their relative incubation period in each experiment, the tomato varieties fall into a fairly regular series, in which the S.F. 2 type, Giant Red, and Golden Queen were the most susceptible, and Maincrop, Stirling Castle, and Superlative were among the most resistant (Table II). In general, a fair correlation was observed between relative incubation period and type of infection. In the most susceptible varieties, the lesions frequently covered the entire leaf surface, while in Stirling Castle, for instance, they remained small and sharply defined, frequently localised towards the apex of the leaf. The actual differences in incubation period were greatest at temperatures lower than the optimum for infection, and at high temperatures these differences were very slight, although the differences in type of infection were retained. There was, moreover, some slight evidence that the minimum relative humidity necessary for sporulation was higher in the resistant varieties than in the susceptible. The identity of the causa

Table II

Inoculation experiments with Cladosporium fulvum

Name of plant	Results positive Relative incubation period 1	2	3	4	Results negative
Tomato:					
Giant Red	//	///	—	—	—
Golden Queen	//	///	—	—	—
Kondine Red	/	///	/	—	—
Open Air	—	///	//	—	—
Plentiful	//	/	—	//	—
Earliest of All	—	/	/	//	/
Up-to-Date	—	/	//	/	/
Large Yellow	—	—	///	/	/
Maincrop	—	/	//	—	//
Stirling Castle	—	/	/	—	///
Superlative	—	—	—	///	//
S.F. 1	//	//	—	/	—
S.F. 2	/////	—	—	—	—
S.F. 3	—	—	—	—	/////

Five experiments are recorded, and each mark "/" represents a record in a single experiment. Where the results are positive, column 1 represents the shortest incubation period observed in any one experiment, and column 4 the longest.

organism was confirmed by reisolation to artificial media. The currant tomato, or S.F. 3 type, remained immune, and neither the potato varieties nor any of the other Solanaceae were infected. Of the varieties of tomato investigated, Stirling Castle has been shown to possess a high degree of resistance by Jagger (18), Small (41), and Bewley and Orchard (7) in England, and by Norton (29) and Alexander (3) in the United States. Resistance was also demonstrated for Maincrop by Small (41), Newhall (28), and Alexander (3). On the other hand, Up-to-Date is mentioned as a resistant variety by most workers, while no record of resistance has been found for the variety Superlative. No record is known to exist of the occurrence of *C. fulvum* on any other species than the tomato, *Lycopersicum esculentum*. The immunity of the currant tomato was first recorded by Sengsbusch and Loschakowa-Hasenbusch (40) in 1932, and was confirmed by Osmun (30) and Alexander (3) in the United States in 1934. It is confirmed in the present investigation for Messrs Suttons' currant tomato (the S.F. 3 type), and also for *L. racemigerum* from Kew and from the John Innes Horticultural Institution.

(2) *Experiments with grafted plants*

The preliminary experiments showed that the grafting experiments could be confined to a limited number of plants, differing widely in their reaction to the two diseases under consideration, as shown in Table III.

Table III

	Resistant	Susceptible
Phytophthora infestans	Tomatoes: Maincrop, Stirling Castle All "other plants"	Tomatoes: Giant Red, Golden Queen All potato varieties
Cladosporium fulvum	Tomatoes: Maincrop, Stirling Castle, S.F. 3 All potato varieties All "other plants"	Tomatoes: Giant Red, Golden Queen, S.F. 2

As many different combinations as possible of resistant and susceptible plants were inoculated in each case. The experiments were delayed until new growth had developed from both stock and scion. The plants were kept close for 7 days after inoculation and then (in the case of *Cladosporium fulvum* inoculations) hardened off as much as possible to prevent premature defoliation. Controls were provided throughout; in a few cases only detached leaves had to be used, for lack of other material. The results are presented in tabular form below:

(*a*) *Phytophthora infestans.*

(i) *Stirling Castle and Giant Red tomatoes.* In two grafts, infection was recorded on Giant Red (both stock and scion) after 7 days. The lesions remained small, with scanty aerial mycelium, exactly as in the controls. Stirling Castle likewise remained free from infection.

(ii) *Potato on Maincrop or Stirling Castle tomato.* Five grafts were inoculated, and in every case aerial mycelium was recorded on the potato after 5 days, while the tomato stock remained free from infection. The scions were eventually completely destroyed by the disease.

(iii) *Potato on* Datura stramonium: *potato on* Solanum nigrum. As before, aerial mycelium was recorded on the scion after 5 days, while the stock remained completely free from infection.

(iv) Atropa belladonna *on Giant Red tomato: Giant Red tomato on* Datura stramonium. Normal symptoms were recorded on Giant Red tomato after 8 days and after 7 days respectively. Neither *Atropa belladonna* nor *Datura stramonium* were infected.

(*b*) *Cladosporium fulvum.*

(i) *Giant Red and Stirling Castle tomatoes.* Both varieties behaved exactly as in the controls and in the previous experiments. In two experiments, the incubation periods for Giant Red were 14 and 24 days respectively (depending on differences in average temperature), and for Stirling Castle 20 and 30 days respectively, both stock and scion of each variety behaving alike. Normal differences in type of infection were also observed.

(ii) *S.F.* 2 *on Stirling Castle tomato.* Incubation periods were 13 and 19 days respectively. Eventually, the lower leaves of the scion were completely covered by the fungal lesions, which, in the stock, were confined to the extremities of the apical leaflets.

(iii) *S.F.* 3 *and Giant Red tomato.* In no case was type S.F. 3 infected. Five grafts were inoculated, in three of which this plant was the scion. The results with Giant Red were normal throughout.

(iv) *Potato on Giant Red tomato:* Atropa belladonna *and* Datura stramonium *on Giant Red tomato.* In all three cases, the tomato stock was infected normally, while the scion remained completely immune.

(v) *S.F.* 2 *on* Solanum nigrum. Normal infection of type S.F. 2; *Solanum nigrum* completely immune.

Within the limitation imposed by the absence of exact quantitative data, the results indicate in both cases the complete absence of any reciprocal influence of stock and scion, affecting disease relationships. Both components have been shown to retain their characteristic reaction to infection irrespectively of the manner in which they were employed. This applies equally well to slight varietal differences as well as to the wider, specific host relationships.

IV. Discussion

The value of grafting experiments in the interpretation of disease relationships has been discussed in a recent book by Krenke (22), and brief notice must be made of his observations before proceeding to a consideration of the present experimental work. Krenke distinguished both active and passive immunity from "growth resistance" (*Wachstumresistenz*), depending on period of maturity, or on vigour of growth or regenerative capacity not in specific relation to disease. The more markedly specific and, strictly speaking, genotypic were the resistant properties, the less were they liable to be modified by grafting, and in this connection an important distinction was made between "non-susceptibility" (*Unempfindlichkeit*), or complete genotypic inability to permit infection, and "resistance" (*Widerstandsfähigkeit*), which was also a phenotypic expression capable of modification by environmental factors.

Inoculation experiments with *Phytophthora infestans* showed that it was impossible to distinguish between the different potato varieties, all of which were highly susceptible to infection. On the other hand, the twelve tomato varieties exhibited well-marked differences in susceptibility, with a higher general level of resistance. According to Reddick (33), immunity in the tomato was "derived" in the same way as it was in

Solanum demissum and other entirely immune species, its existence indicating that these species had been in contact with *Phytophthora infestans* for a long period of time. Moreover, the more uniform susceptibility of the potato varieties was due to the fact that *Solanum tuberosum* was a comparatively recent introduction from a region (*i.e.* South America) in which the fungus did not occur. Even in the potato, the existence of a certain degree of active immunity seems probable (13), although in some cases this appears to be supplemented by morphological and other properties of a purely passive nature (24). While derived immunity is essentially genotypic and is not affected by environmental conditions, it is not comparable to "non-susceptibility", which should be restricted to a complete primary immunity, in no sense derived from the condition of susceptibility. The grafting experiments were confined to potato, resistant and susceptible varieties of tomato, the highly resistant *Solanum nigrum*, and *Atropa belladonna* and *Datura stramonium*. The results showed that the characteristic reaction of stock and scion were entirely unaltered by grafting. In Volk's (43) experiments, slight differences in susceptibility were recorded for potato scions when grafted on tomato (immune), and these were attributed to differences in the length of the vegetative period. However, these observations were confined to individual leaves in the vicinity of the graft union. In the present investigation, the infection was estimated over whole shoots, most of which had developed subsequently to grafting and under identical environmental conditions. Accordingly, the results fully corroborate the suggestion already put forward, namely, that resistance and susceptibility to *Phytophthora infestans* are essentially genotypic properties. Grafting experiments afford no evidence of whether immunity is derived or not, neither do they distinguish between the active and passive character of resistance. Certainly, in the species investigated, if resistance is passive in character, the properties on which it depends are not such as are capable of effective transmission from stock to scion or *vice versa*.

Inoculation experiments with *Cladosporium fulvum* revealed well-marked differences in susceptibility among the twelve tomato varieties, shown both by incubation period and by intensity of infection. No varieties, however, were entirely immune. The true nature of resistance remains unknown. *C. fulvum* is apparently strongly specialised to the tomato (*Lycopersicum esculentum*), and there is no satisfactory evidence for the existence of distinct strains differing in cultural characteristics, host relationships, or geographic distribution. The immunity of the currant tomato (*L. racemigerum* Lange or *L. pimpinellifolium* Dun.) is fully confirmed, but Schmidt's opinion (39), that it depends on the presence of a water-soluble substance termed "prohibitin", capable of inhibiting spore germination, has received no support. Further, Schmidt's own figure illustrating the morphology of *Cladosporium fulvum* bears no resemblance to the normal appearance of the fungus, and does not agree with the accepted diagnosis of the genus. It suggests, in fact, the contamination of his cultures with *Trichothecium roseum* Lk., a view that is also supported by his account of their behaviour on artificial media. The grafting experiments permit of a similar interpretation to that adopted in the case of *Phytophthora infestans*. The results showed the complete absence of any effect of grafting on disease relationships, either in resistant and susceptible varieties of tomato, or in the immune currant tomato or any other plants investigated. Resistance to *Cladosporium fulvum* appears to be essentially genotypic in character. Certainly, the almost continuous variation exhibited between the different varieties suggests that it has been "derived" in the manner proposed for the origin of resistance to the "potato strain" of *Phytophthora infestans*. From the manner of its inheritance, it would appear to be related to the complete immunity of the currant tomato (40), but direct evidence is lacking. In all probability, the immunity of the potato and other genera represents an example of genuine and complete "non-susceptibility".

Negative results cannot be conclusive in themselves, and therefore the grafting experiments can be of little value except as circumstantial evidence in support of conclusions previously derived from other considerations. In the present discussion, attention has been directed towards an understanding of the evolutionary significance of parasitism. In both genera of fungi, the presence or absence of biological specialisation must be related to the probable past history of the disease, and the host range and cultural characteristics of related species must be considered in terms of the value of their systematic position. On the one hand, immunity may be found to have been derived from the susceptible condition common to a large number of related genera; on the other hand, the origin of susceptibility may be found in the progressive specialisation of parasitism from a wide range of saprophytic or weakly parasitic activities.

V. SUMMARY

Experiments are described which are concerned with the possibility of an increase or decrease in susceptibility to pathogenic fungi, induced or transmitted by grafting. Two diseases are investigated, namely, potato

blight, caused by *Phytophthora infestans* (Mont.) de By., and tomato leaf mould, caused by *Cladosporium fulvum* Cke.

Preliminary inoculation experiments were carried out on eleven large-fruited varieties of tomato, eight varieties of potato, various small-fruited tomatoes, and other Solanaceous plants, with the following results:

Phytophthora infestans. All the varieties of potato were equally susceptible, but certain varieties only of tomato, viz. Giant Red, Golden Queen. Detached leaves of the currant tomato were also infected.

Cladosporium fulvum. Only tomato varieties and certain small-fruited tomatoes were susceptible. Giant Red and Golden Queen were particularly susceptible, Stirling Castle and Maincrop were relatively resistant. The currant tomato (*Lycopersicum racemigerum* Lange or *L. pimpinellifolium* Dun.) was immune. No support can be given to Schmidt's suggestion that immunity depended on the presence of a substance termed "prohibitin", capable of inhibiting spore germination. It is suggested that the identity of Schmidt's cultures was mistaken.

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About fifty grafts were made involving various combinations of resistant and susceptible plants. The grafts were inoculated, in most cases, after new growth had developed from stock and scion. Without exception, the results of these experiments were the same as on the ungrafted material, both stock and scion retaining their characteristic reaction to infection unaltered. Although negative results cannot be conclusive in themselves, they indicate that resistance and susceptibility are either genotypic properties of the protoplasm or else are due to some factor that is not, as such, transmissible from stock to scion or *vice versa*.

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GLOMERELLA PHACIDIOMORPHA (CES.) PET. ON *PHORMIUM TENAX* IN BRITAIN

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(With Plate I and 2 Text-figures)

INTRODUCTION

PHORMIUM TENAX, the New Zealand flax, though native only in the southern hemisphere, has been grown in England since its introduction into Kew Gardens in 1789. Since then various attempts have been made to cultivate the plant on a commercial scale, and success has been achieved in such widely different localities as the north-west of Scotland, the south-west of England and the south-west of Ireland. No outstanding success has, however, been achieved in the establishment of a *Phormium*-growing industry. The most recent attempt was made on a farm in Devonshire, where, during the few years preceding 1934, large acreages of the crop were grown. In the spring of 1934 a portion of this crop showed symptoms of ill-health. Samples of the diseased material were received in March of the same year.

SYMPTOMS OF THE DISEASE

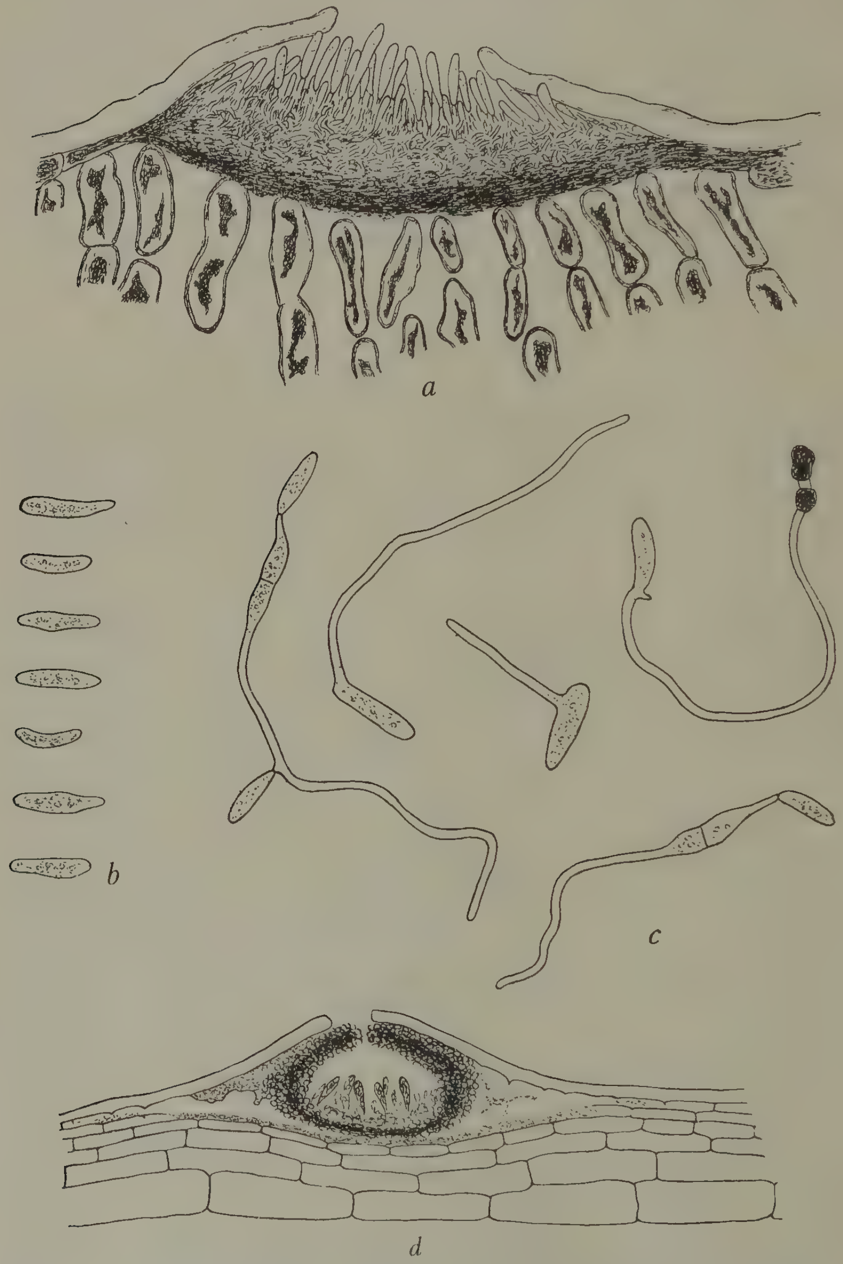
The symptoms were confined to a small portion of the crop, which appears to have been neglected since the bases of the *Phormium* plants were smothered in a dense mat of weeds. The leaves show two distinct types of infection. In the first type oval patches of dead tissue appear on otherwise apparently healthy leaves. The centre of the patch is bleached a dirty grey, which gradually merges into a brownish grey towards the outside. A reddish brown margin separates infected from healthy tissue. This localised type of infection is more frequent on the younger leaves of the fan, though not on the very young inner leaves. In the second type of infection whole leaves are affected. They die and turn a greyish brown colour, often rolling throughout their length, forming the "leaf tubes" familiar to New Zealand cultivators. The cuticle peels off in large flakes, exposing fungus fructifications, which in places give the leaf a jet black appearance. Decay of the tissues is always more advanced towards the tips, and it appears that infection starts at the tip and spreads downwards. Early stages of infection were observed, in which the leaves bore only a few fructifications towards the tip. Many leaves are dead near the tip, but show no sign of parasitic attack, and it is difficult to determine how far the symptoms are due to a parasite and how far to poor conditions of growth. This general infection is more obvious on the older leaves of the plant, while here again the very young inner leaves of the fan are unaffected. In addition to the symptoms described signs of attack by burrowing and biting insects were observed on both healthy and infected leaves.

FUNGI ASSOCIATED WITH THE SYMPTOMS

Conidial acervuli and perithecia are abundant on the dead parts of the leaves. On many of the lesions acervuli or perithecia occur alone, while on others the two are intimately mixed. Fructifications are developed on both surfaces of the leaf, though they are more frequent on the upper side, where on small lesions they occur exclusively.

(*a*) *Acervuli*

The positions of acervuli are marked by small, rounded or elongated, light-coloured patches on the surface of the leaf. The acervuli (Text-fig. 1 *a*) are typically *Gloeosporium*-like in section, measuring 200–400 μ across. When very numerous they occur close together, touching each other, and several may join to form a long streak-like pustule, running longitudinally between the veins of the leaf. Acervuli do not occur directly above the veins. They are developed within the epidermal cells, which later become completely disorganised. The basal stroma is made up of brown-black, irregularly rounded cells and is situated in the lower part of the epidermis. On the upper surface the stroma gives place gradually to a layer of more or less rectangular cells, elongated in a vertical direction and gradually becoming lighter in colour. The uppermost cells of this layer grow apart and form a hymenium of numerous conidiophores, cylindrical, hyaline, thin-walled, with few septa and about 20 μ long, each bearing a solitary, terminal conidium. The conidia (Text-fig. 1 *b*) are one-celled, hyaline, slightly granular, thin-walled, $19–26 \times 5{\cdot}5–7{\cdot}5\,\mu$, and vary in shape from subclavate, cylindrical to ovoid-elliptical, occasionally irregularly curved and usually flattened at the point of attachment. They are produced abundantly and at maturity force open the resistant cuticle, which opens in a jagged, more or less elongated slit. The conidia are extruded in an orange-red cushion-shaped mass.

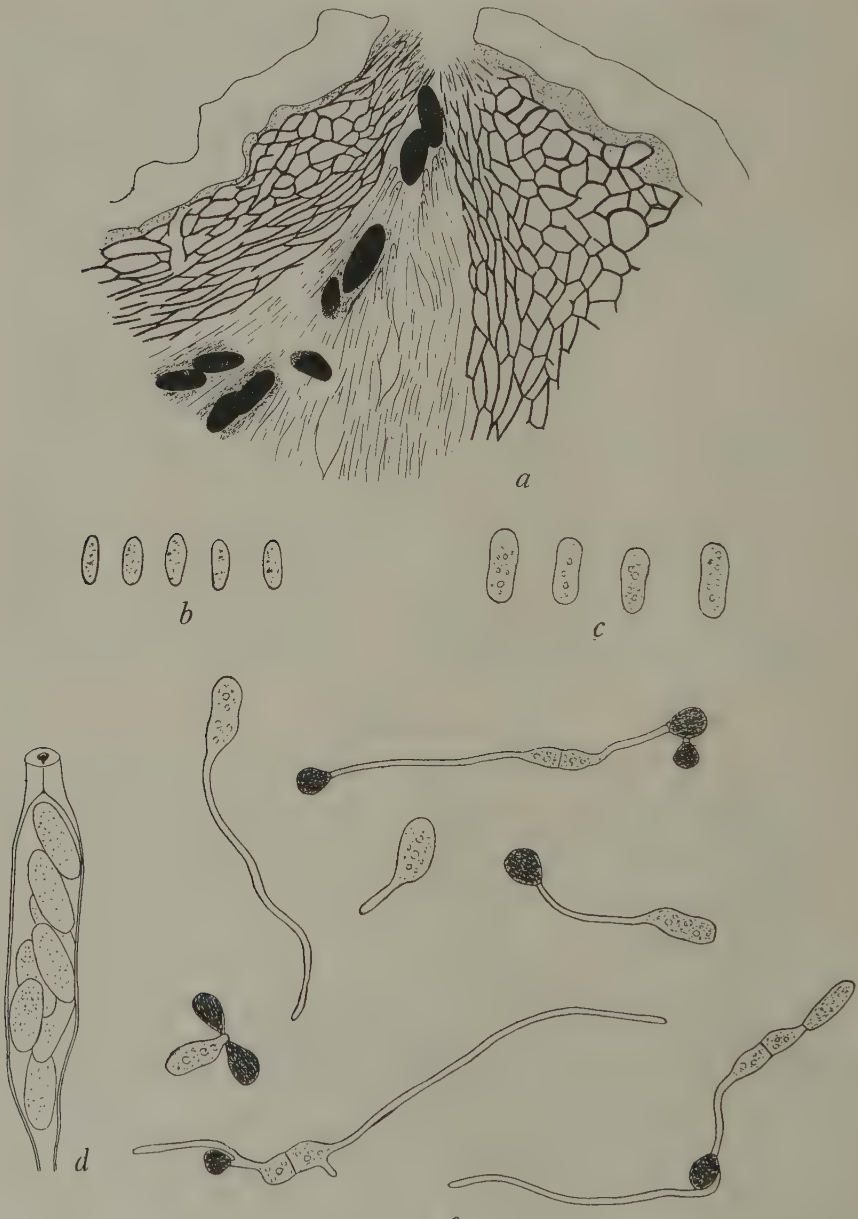


Text-fig. 1. *G. phacidiomorpha.* (*a*) Transverse section. Acervulus; × 530. (*b*) Conidia; × 530. (*c*) Conidia germinating; × 530. (*d*) Perithecium on upper surface of leaf; × 90.

The conidial stage described was identified as belonging to a fungus *Cryptosporium rhodocyclum* Mont. ((11), XXII, 1235), at one time supposed to be a conidial stage of one of the Dothidiales. The fungus was first recorded from Lisbon, under the name of *Phyllosticta haematocycla* Berk., and was later found in the Botanic Garden at Ajuda, Portugal. The above description agrees also with that of *Fusarium Phormii* P. Henn. ((11), XVI, 1101), a fungus found by Hennings at the Berlin Botanic Garden on *Phormium tenax* in association with *Physalospora Phormii* (2). It may, therefore, be concluded that the present fungus is identical with both *Fusarium Phormii* P. Henn. and *Cryptosporium rhodocyclum* Mont. Pfaff found it occurring on the same host at Bozen in the Tyrol (9). *Fusarium Phormii* was later placed in the genus *Gloeosporium* and, finally, von Höhnel named it *Gloeosporidium rhodocyclum* (Mont.) v. Höhn. He considered it identical with *Cryptosporium rhodocyclum* Mont. Petrak (9) re-examined the fungus from dried material and pronounced it a typical *Colletotrichum* without the formation of border setae. He named the fungus *C. rhodocyclum* (Mont.) Pet., but did not definitely identify it with *Cryptosporium rhodocyclum* Mont. It appears, however, that the two are identical. Thus the fungus under consideration is now named *Colletotrichum rhodocyclum* (Mont.) Pet., and has been known under the various synonyms mentioned above.

(*b*) *Perithecia*

The perithecia occur all over the dead leaves, usually crowded together, often touching each other, or fused into groups of two or three. They occur on both surfaces of the leaf and their position in the tissues varies with the surface on which they occur. Above, they develop within the epidermal cells, while below they are embedded in the deeper tissues of the leaf (Plate I, fig. 1 and Text-fig. 1 *d*). The perithecia above are necessarily flattened, since they occupy only the space between the bulging cuticle and the first layer of a resistant hypoderm; those below are more or less globose. Single perithecia measure 200–300 μ in diameter, slightly less in depth. The wall of the perithecium is membranaceous, becoming brittle with age and is made up of several layers of dark brown cells, net-like in appearance. It is slightly thicker towards the sides than above and below, and it projects above in a beak-like ostiole. There is no definite stroma, unless it be represented by a development of branched hyphal strands, radiating from the walls of the perithecium and penetrating far into the epidermal layer. Near the perithecia the cells of the epidermis are filled with such dark brown hyphae. In the case of peri-



Text-fig. 2. *G. phacidiomorpha.* (*a*) Transverse section. Mouth of perithecium showing ejection of ascospores; ×850. (*b*) Ascospores; ×530. (*c*) Ascospores swollen before germination; ×530. (*d*) Ascus; ×850. (*e*) Ascospores germinating; ×530.

thecia developed in the lower side of the leaf these hyphal strands penetrate deep into the assimilating tissue, while on the upper side they do not normally or only seldom penetrate the hypodermal layers of the leaf. Inside the perithecium the wall gradually changes into a layer of hyaline cells, specially well developed near the mouth of the perithecium. The asci (Text-fig. 2 *d*) are numerous, all at one level, sessile or shortly stalked, cylindrical or spindle-shaped, slightly swollen, tapering below into a short stalk and with a truncate apex, which is thickened and traversed by a narrow pore. They measure $50\text{–}70 \times 10\text{–}15\,\mu$, but vary greatly according to the stage of development. Towards maturity they elongate and occupy the upper part of the perithecium. The ascospores (Text-fig. 2 *b* and *c*) are eight to each ascus, arranged irregularly in two rows, ovoid-elliptical to oblong, seldom irregular, one-celled, faintly greenish coloured, granular with usually one central oil drop, and measure $12\text{–}15 \times 5\text{–}6\,\mu$ when ripe and dry. When soaked in water they elongate and become swollen at the ends, measuring $14\text{–}22\,\mu$ long, and becoming constricted in the middle. Slender, thread-like paraphyses are present in the young perithecium, but these become mucilaginous as the asci ripen.

The perithecia belong to the fungus *Glomerella phacidiomorpha* (Ces.) Pet. This species was first described as *Sphaeria phacidiomorpha* Ces. and, later, erroneously named *Didymella phacidiomorpha* (Ces.) Sacc. ((11), I, 539). In 1889, Hennings referred to the fungus as *Physalospora Phormii*, but his report was not published, and, later, Schröeter named the species *P. Phormii* Schröet. ((7); (11), XI, 292). Theissen (12) in a revision of the genus *Physalospora*, created a new genus, *Hypostegium*, and renamed the species *H. Phormii* (Schröet.) Theiss. Petrak (10) re-examined the fungus and, after pointing out that it was incorrectly assigned to the genus *Catacauma* by von Höhnel as *C. Phormii*, placed it in the genus *Glomerella* as *G. phacidiomorpha* (Ces.) Pet.

The fungus appears to be widely distributed. It has been collected from various places in Germany and has recently been recorded from Latvia (14) and Kenya (15). It has not been previously recorded from Britain. As already stated, Hennings found the fungus in the Plant Houses at the Berlin Botanic Garden in 1889, occurring as a dangerous parasite on *Phormium tenax*. Kratz (7) in 1907 published an account of the fungus, dealing almost exclusively with the mycelium within the host and its effect on the different tissues of the leaf. Later publications have dealt mainly with the systematic position of the fungus.

In all accounts previous to that of Petrak in 1927 no conidial stage is definitely assigned to the fungus, though Hennings records having found it in association with *Fusarium Phormii* and suggests a possible relationship. *F. Phormii* has since been named *Colletotrichum rhodocyclum* (Mont.) Pet., and in 1927 Petrak refers to this fungus as the conidial stage of *Glomerella phacidiomorpha* (Ces.) Pet. His chief reason for placing the perfect stage in the genus *Glomerella* is, in fact, that its imperfect stage is a *Colletotrichum*. It does not appear, however, that any cultural work has been done in order to prove the relationship between the two stages. In the present case it was undertaken with this end in view.

CULTURAL CHARACTERISTICS

(1) *Germination of the spores*

(*a*) *Ascospores of* Glomerella phacidiomorpha.

Material with ripe perithecia was placed in a damp chamber, and within 24 hours abundant ascospores were shot out on to a cover-glass placed 5 mm. above the perithecia; later experiments showed that they may be ejected to a distance of 1·5 cm. Microtome sections prepared from such material showed ascospores passing through the ostiole of the perithecium, one at a time. Tips of asci were observed near the ostiole, though it was impossible to distinguish them in the ostiole itself, while unripe asci were confined to the lower part of the perithecium. Thus it appears that as the asci ripen they elongate till their tips reach the ostiole. Groups of ascospores, representing the contents of single asci, were not found on the cover-glass, but the spores were scattered irregularly. This, taken in conjunction with the facts that the pore through the thickened apex is very narrow, and that the spores are found in sequence in the ostiole of the perithecium, appears to indicate that the spores are ejected singly from the ascus (see Text-fig. 2 *a*).

The ascospores germinate readily in pure water within 4 or 5 hours of ejection. They increase in size and become markedly constricted in the middle just before germination. One or more terminal or lateral germ tubes are formed and a septum forms in many of the spores, particularly in those with two germ tubes. Appressoria are produced in abundance on the young mycelium. They are dark structures, terminal or lateral, at first spherical and later becoming very irregular. Within 3 or 4 days conidia, exactly similar to those of *Colletotrichum rhodocyclum* already described, are budded off from the young mycelium. A later stage in germination shows a much-branched, septate mycelium and numerous conidia and irregular appressoria (Text-fig. 2 *e*).

(*b*) *Conidia of* Colletotrichum rhodocyclum.

The conidia germinate in pure water, but more readily in 1 per cent. glucose solution or in a weak extract of *Phormium* leaves. Appressoria are formed, similar to those on the young mycelium of *Glomerella phacidiomorpha*, though in this case they are not so abundant. After 3 or 4 days the mycelium buds off secondary conidia, borne terminally or laterally on the hyphae (Text-fig. 1 *c*).

(2) *Cultures on artificial media*

Cultures derived from single ascospores of *Glomerella phacidiomorpha* and from single conidia of *Colletotrichum rhodocyclum* were grown on malt agar, oat agar and *Phormium* leaf extract agar, and on pieces of killed, sterilised *Phormium* leaf.

On malt agar the culture of *Glomerella phacidiomorpha* grows rapidly over the surface of the medium, at first producing only a very scanty aerial growth. During the first week the mycelium is colourless or greyish white, but at the end of 2 weeks plate cultures show a distinct concentric arrangement of greenish brown rings within the medium, while the aerial mycelium turns distinctly grey. Soon after a deep brown colour develops in the centre of the plate. In 3 weeks the whole culture is an even greenish brown, which gradually darkens to a greenish black and remains until the culture dries. The dark colour is due to the development of a tough, compact layer of dark, thick-walled hyphae immediately under the surface of the medium. After 4 or 5 weeks numerous dark specks appear in the culture. They are of the nature of stromata and are formed by the aggregation of the subaerial hyphae into dense knots. These stromata increase in size up to 1–2 mm. in diameter and give rise to dense, white tufts of erect, branched hyphae, bearing conidia similar in size and form to those of *Colletotrichum rhodocyclum*. The basal stroma with its tuft of erect hyphae is, in fact, an indefinite conidial acervulus of *C. rhodocyclum*. No perithecia were observed in malt agar cultures.

Cultures from ascospores grown on oat agar are exactly similar to those on malt. On *Phormium* leaf extract, agar growth is slow and scanty. No colour is developed and no fructifications have been observed in the extract agar cultures.

When mycelium of *Glomerella phacidiomorpha* is placed on the wounded surface of killed, sterilised *Phormium* leaf a dense white aerial growth is produced. The leaf tissues are permeated by thick, dark brown hyphae, which in places aggregate into dark stromata, pallisade-like in section, and giving rise to typical *Colletotrichum rhodocyclum* acervuli.

Perithecia of *Glomerella phacidiomorpha* are developed a day or two later.

On malt agar the mycelium of *Colletotrichum rhodocyclum* spreads rapidly and the cultures are similar to those of *Glomerella phacidiomorpha* on the same medium. Conidia are reproduced after 3 weeks, not in loose tuft-like fructifications, but in compact circular acervuli. Drops of liquid form near the acervuli and the conidia are extruded in an orange-red mass. Sectoring occurs frequently in some of the cultures (Plate I, fig. 3). Subculturing from such sectors gives cultures with smaller acervuli than normal, and with less aerial growth.

On oat agar acervuli are produced in abundance and the growth is similar to that on malt. On *Phormium* leaf extract agar development is slow and fructifications are never produced. Conidia germinate readily on the wounded surface of killed, sterile *Phormium* leaf. Aerial growth is scanty, but the leaf tissues are rapidly permeated by a mycelium of thick, dark brown hyphae. Numerous dark stromata are produced under the leaf epidermis. They are composed of thick brown hyphae, arranged in a pallisade-like manner at right angles to the leaf surface. On the part of the leaf from which the epidermis was removed some of the thick brown hyphae project upwards to form the typical border setae of the genus *Colletotrichum*. Conidial acervuli are found on the stromata. Perithecia of *Glomerella phacidiomorpha* are developed on the leaf 3 or 4 weeks after inoculation. The occurrence of border setae on that part of the leaf with the epidermis removed is interesting, since Petrak, in his identification of the imperfect stage as a typical *Colletotrichum* without the formation of border setae, says that these structures are only suppressed due to the fact that the acervuli are developed below the epidermis with its thick cuticle. Their occurrence on the part of the leaf with no cuticle confirms this view.

RELATION BETWEEN THE TWO FUNGI

The cultural experiments prove that *Colletotrichum rhodocyclum* (Mont.) Pet. is the imperfect stage of *Glomerella phacidiomorpha* (Ces.) Pet. This statement is founded on the following facts:

(1) On sterile *Phormium* leaf mycelium derived from a single ascospore of *Glomerella phacidiomorpha* gives rise to acervuli of *Colletotrichum rhodocyclum*, then to perithecia of *Glomerella phacidiomorpha*.

(2) On sterile leaf conidia of *Colletotrichum rhodocyclum* give rise to a mycelium which reproduces conidia and gives rise to perithecia of *Glomerella phacidiomorpha*.

(3) Germinating ascospores and the young mycelium of *Glomerella phacidiomorpha* bud off conidia similar to those of *Colletotrichum rhodocyclum.*

(4) Cultures of *Glomerella phacidiomorpha* on malt agar give rise to fructifications of *Colletotrichum rhodocyclum.*

A description of *Glomerella phacidiomorpha* with its conidial stage is as follows:

Glomerella phacidiomorpha (Ces.) Pet.

Sphaeria phacidiomorpha Ces., *Didymella phacidiomorpha* (Ces.) Sacc., *Physalospora Phormii* Schröet., *Hypostegium Phormii* (Schröet.) Theiss., *Catacauma Phormii* (Schröet) v. Höhn.; *Cryptosporium rhodocyclum* Mont., *Phyllosticta haematocycla* Berk., *Fusarium Phormii* P. Henn., *Gloeosporidium rhodocyclum* (Mont.) v. Höhn., *Colletotrichum rhodocyclum* (Mont.) Pet.

Perithecia solitary or two or three together, in two positions, (*a*) on the upper surface of the leaf, between the epidermis and the hypoderm, compressed from top to bottom, (*b*) on the lower surface, embedded in chlorenchyma, spherical; necks short, slightly protruding at maturity; wall of several layers, dark brown to black, membranaceous, later becoming brittle. Perithecia 200–300 μ across. Asci numerous, spindle-shaped or cylindrical, slightly swollen, with thickened apices, $64–75 \times 10–15 \mu$. Ascospores irregularly in two rows, one-celled, ovoid to oblong, hyaline, slightly granular, with central oil drop, $14–22 \times 5–6 \mu$. Paraphyses slender, thread-like, becoming mucilaginous.

Acervuli numerous, circular, often joined in rows, bursting through the epidermis at maturity. Conidiophores crowded, cylindrical, about 20 μ long. Conidia solitary, terminal, one-celled, cylindrical or ovoid-elliptical, sometimes irregular, hyaline, slightly granular, usually with one central oil drop, $19–26 \times 5{\cdot}5–7{\cdot}5 \mu$. Conidial cushions orange-red.

POSITION OF THE PERITHECIA IN *GLOMERELLA PHACIDIOMORPHA*

The position of the perithecia in *G. phacidiomorpha* is interesting. It has been proved as far as is possible without culturing from individual fructifications that the superficial and the embedded perithecia belong to the same species. The difference in position is, normally, a constant one, depending on the surface of the leaf on which the perithecia occur and seems to be correlated with the marked difference in anatomical structure between the upper and lower surfaces of the leaf. Assuming that the perithecia occur near the surface in response to a definite stimulus, *e.g.* a supply of air, and that the fungus mycelium is present in both the upper and lower parts of the leaf, the perithecial fundaments will form on both sides of the leaf, just below the surface. The assimilating cells in the lower part of the leaf are readily disorganised and are forced apart by the developing perithecium, which grows down into the tissues. On the upper surface, however, the hypoderm is resistant, so that the perithecia are confined to the space between the cuticle and the first layer of hypoderm.

On artificially infected leaves kept under a bell-jar, perithecia are, however, developed deep in the hypodermal layer on the upper side of the leaf (see Plate I, fig. 4). Such perithecia are markedly constricted in the middle and seem to be developed under lateral pressure from the hypodermal cells. In this case it seems probable that the conditions of heat and saturation of moisture under which the leaf is kept so lowers the resistance of the host tissues as to allow of the partial disorganisation and penetration of the hypoderm. It is interesting to note that Kratz (7) describes the perithecia of *G. phacidiomorpha* as occurring immediately under the epidermis, but figures one deeply sunk in the hypoderm. Possibly correlated with this is the fact that his observations were made on *Phormium tenax* grown under glass in conditions approximating those under which the above leaves were kept.

This variation in position of the perithecium within one species raises the question of the value of fructification position as a taxonomic character in the fungi. The "strictly sub-epidermal position" of the perithecia of *Glomerella phacidiomorpha* was used by Theissen to distinguish his new genus *Hypostegium* from the genus *Physalospora* (12). Other genera, too, have been subdivided on the basis of the position of the fructification in the host tissues. *Lophodermium* (3, 4) has been subdivided into several genera, according to whether their apothecia are subcuticular, subepidermal or deeper in the tissues. The occurrence of perithecia of *Glomerella phacidiomorpha* in two distinct positions, together with the fact that slight changes in the environment of the host appear to cause variations in the position of the perithecia indicates that in many cases this character may be of singularly little value in taxonomy.

INOCULATION EXPERIMENTS

(1) *Inoculations on the entire plant*

Apparently healthy plants, derived from division of the root-stock and potted in April 1934, were used for the experiments. The older leaves were cut off in order to stimulate new growth and to get rid of all dead tissue, for *Phormium* leaves die naturally when just over 2½ years old.

It was found later that the plot of *Phormium* from which the experimental plants were derived was infected with *Glomerella phacidiomorpha*. No infection was noticed at the time of potting, and it is possible that the plants in the plot were infected later. At any rate the young growth on the experimental plants was entirely free from the fungus at the time of inoculation. In June, leaves of different ages were inoculated. Mycelium derived from a single ascospore was placed in a scalpel wound in the leaf, and the whole wrapped in cotton-wool. Controls with similar wounds but with no mycelium were set up. By December none of the control wounds showed any sign of infection. The inoculated wounds, on the other hand, developed a bright red colour, which spread longitudinally and laterally so as to form an oval patch with a bleached centre and a bright red margin. By October the lesions were on an average $2 \times 1\frac{1}{2}$ in. and had ceased to spread. Three out of eleven bore perithecia. Inoculations on healthy tissue produced only this localised type of infection in the leaves.

Suspensions of conidia on the uninjured surface of a healthy leaf failed to bring about infection.

(2) *Inoculations on detached leaves*

Cut leaves under a bell-jar and pieces of leaf in Petri dishes were used. Suspensions of conidia on the uninjured surface of the leaf failed to produce infection. After fixing and staining the part of the leaf inoculated, the germinated conidia were easily observed. Long straggling germ tubes with numerous appressoria were produced, but in no case were they observed to penetrate the cuticle or pass into the stomata. Suspensions of conidia on the uninjured surface of a killed *Phormium* leaf do not bring about infection. It seems, therefore, that the germ tubes are incapable of penetrating the thick cuticle of the leaf.

(3) *Observations on natural infections*

Further evidence as to the virulence of the fungus was obtained from observation of natural infections. The plants used for inoculation, though apparently healthy when potted, later showed signs of die-back towards the tips of the leaves, caused by the check received at the time of potting. The leaf tips split longitudinally along the midribs. In October, when fungus fructifications were observed on the experimental inoculations, many of the leaf-tips were obviously infected and bearing both acervuli and perithecia. Many of the lesions seemed to spread longitudinally along the split mid-rib, and it is concluded that infection took place in this area. The source of the spores has not been determined, but they may have come from the infected plants in the plot or from the accidental scattering of the conidia used in the experimental inoculations. Similar infection of the dying tips was observed on detached leaves under controlled conditions. A young leaf, showing no sign of infection, was sterilised on the surface and inoculated on both the upper and lower surfaces, and kept under a bell-jar. The experimental inoculations were, as before, unsuccessful, but infection developed on the yellowing leaf-tips and spread downwards as the leaf died. Acervuli were produced on the dead tissue, and fungal hyphae were abundant in the yellowing region.

PATHOGENICITY OF THE FUNGUS

Hennings (2) describing the fungus as he found it in the plant houses of the Berlin Botanic Garden, stated that it is a dangerous parasite, producing large brown spots on the *Phormium* leaves, especially towards the tips. Later, he said, the whole leaf tends to die.

Kratz (7) stated that the germ tubes do not enter through the stomata, but must do so either through wounds or by penetration of the cuticle. He describes how the mycelium attacks the various tissues of the leaf. The central water-storage tissue is least resistant, while the fibres are scarcely, if ever, attacked. At first an intercellular mycelium with haustoria and later an intracellular mycelium is formed.

The results of present inoculation experiments show that the fungus is only weakly parasitic. That it is more than a saprophyte is proved by its ability to produce a lesion on healthy tissue. The spores do not seem, however, to be able to penetrate healthy uninjured leaves. It appears that the germ tubes enter through wounds, and that the fungus is capable of extensive growth only in an already weakened host. The symptoms observed on the original material can be explained on this principle. It seems that in the infected crop the plants were in very bad health, due no doubt to their neglected condition. The resistance of the whole plant was lowered and the leaves themselves were suffering from die-back, with consequent splitting of the leaf-tips. Invasion of such leaves at the tip seems to account for the general infection observed in the plants. Insect injury on the young, more vigorous leaves seems to account for the more localised type of infection.

ECONOMIC IMPORTANCE

The fungus, though only weakly parasitic, may be dangerous. If the spasmodic attempts to cultivate *Phormium tenax* in Britain ever lead to an establishment of the industry, the fungus seems to be a potential source of danger to crops of lowered vitality. Such a condition may be brought about by drought and neglect of proper cultivation. At present, however, the disease is of little economic importance in Britain. The imperfect stage of the fungus, under the name *Fusarium Phormii* P. Henn., has been recorded from New Zealand, where *Phormium* is grown extensively for its fibre ((1), p. 48). It is described as causing large blotches on the leaves, but does not seem to have been investigated, though recognised as a destructive fungus. Until comparatively recently the supplies of *Phormium* were obtained solely by cutting the virgin swamps, and it is only since the start of cultivation that notice has been directed to the parasites of *P. tenax*. Such notice has, so far, been chiefly concerned with the insect pests of the plant.

SUMMARY

1. *Glomerella phacidiomorpha* (Ces.) Pet. has been found to be the cause of a disease of *Phormium tenax* in England. This is the first record of the fungus in Britain.

2. The fungus is only weakly parasitic, and is of danger only to plants of lowered vitality.

3. The imperfect fungus *Colletotrichum rhodocyclum* (Mont.) Pet. is shown to be a conidial stage of *Glomerella phacidiomorpha*.

I wish to express my indebtedness to Mrs N. L. Alcock, Plant Pathologist to the Department of Agriculture for Scotland, who supplied the material of *Phormium tenax*, and to Dr M. Wilson, Mycology Department, University of Edinburgh, who supervised the work.

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EXPLANATION OF PLATE I

Fig. 1. Longitudinal section of leaf of *Phormium tenax*, showing perithecia of *Glomerella phacidiomorpha* in two positions. × 60.

Fig. 2. Infection lesion on leaf of *Phormium tenax*. Nat. size.

Fig. 3. 3 weeks old culture of *Glomerella phacidiomorpha*, showing sectors. Slightly reduced.

Fig. 4. Longitudinal section of leaf of *Phormium tenax*, artificially infected, showing one normal perithecium and one constricted perithecium of *Glomerella phacidiomorpha*. × 60.

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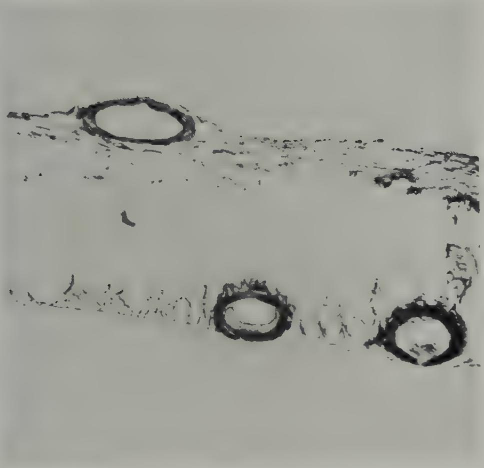
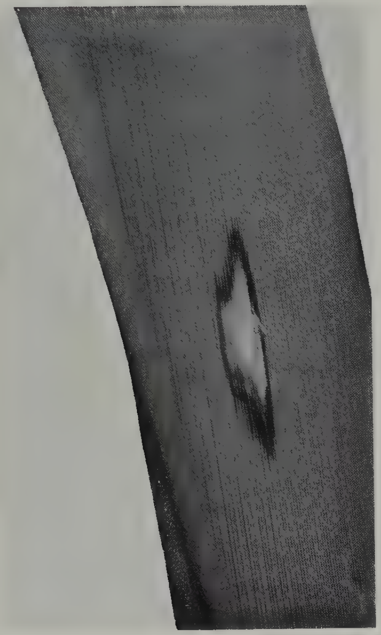


Fig. 1.



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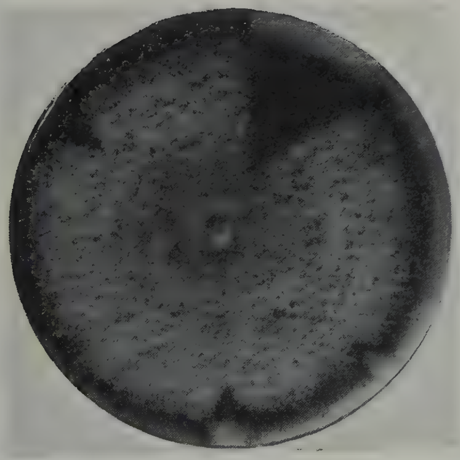

Fig. 3.

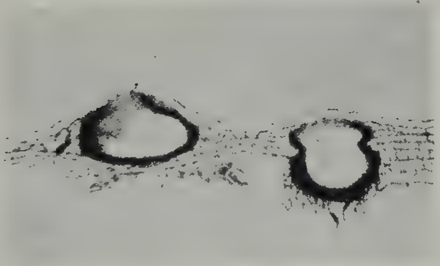

Fig. 4.

KINGHORN.—*Glomerella phacidiomorpha* (Ces.) Pet. on *Phormium tenax* in Britain (pp. 30–44).

BIOLOGIC STRAINS OF *OPHIOBOLUS GRAMINIS* SACC.

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(With 2 Text-figures)

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I. INTRODUCTION

VAN DER LAAR (12) studied *Ophiobolus graminis* Sacc. and *O. herpotrichus* (Fr.) Sacc. in pot experiments in an attempt to differentiate them into strains on a basis of host reaction. He proved the existence of two strains of *O. herpotrichus*, both of which could attack Bocumer winter barley, whereas only one of them infected Wilhelmina wheat. He found no such differentiation in the case of *O. graminis*. It is to be noted that the measure of differentiation was a very broad one, namely, susceptibility or immunity of the host. No account was taken of the degree of injury produced, so that possible quantitative differences were not observed.

As indicated by the work of Kirby (6) *O. graminis* has a wide host range amongst the Gramineae, many genera being susceptible. Padwick and Henry (9) likewise found that the fungus has a wide distribution in this family, although there were noticeable differences between the reactions of the various grasses studied. In a fungus of such broad capabilities as a parasite one might suspect a lack of specialisation or division into biologic strains.

Russel (11) conducted extensive tests in pots of sterilised soil and in the field, using heavy inoculations with *O. graminis* grown on oat hulls. A hundred varieties of wheat belonging to eight subspecies of *Triticum* were all very susceptible to this fungus. Different isolates varied greatly in their ability to attack wheat, and certain isolates when tested at different times varied considerably in their pathogenicity. It is important to note that the infection conditions were decidedly favourable for the parasite, with the result that only highly resistant varieties could be expected to survive.

Krebs (7) discovered two strains of *O. graminis*, one having a temperature optimum for infection at 12–15° C., and the other at 24° C.

Other characteristics besides pathogenicity may be of importance from a practical standpoint. Ability to grow or survive in various conditions of soil temperature, moisture, acidity, aeration, etc., undoubtedly play an important part in the life history of this fungus, and some of these points are studied here.

II. EXPERIMENTAL

(*a*) *Materials*

The author is indebted to Dr Henry of Alberta and Mr Garrett for their assistance in supplying isolates of *O. graminis* from which hyphal tip cultures were made (see Table I).

Table I

Isolate No.	Cultures used in experiments	
	Original inoculum	Source of culture
2	Original isolate of mycelium	*Triticum vulgare*, Canada
3	,,	,, ,,
4	,,	,, ,,
5	,,	,, ,,
6	,,	,, ,,
7	,,	,, ,,
4 A	Hyphal tip	,, ,,
8 A	,,	,, ,,
102 A	,,	*Agropyron tenerum*, Canada
108 A	,,	*A. repens*, Canada
201 A	,,	*Triticum vulgare*, England
202 A	,,	,, ,,
205 B	Single ascospore	,, ,,
302	Hyphal tip	*Hordeum murinum*, Australia

These stock cultures were kept on potato dextrose agar.

The following nutrient media were used, with 20 gm. of agar per litre; in each case the figures represent grams per litre:

(1) *Carrot dextrose*: extract of 200 gm. of steamed carrots; glucose 20.

(2) *Potato dextrose*: similar to (1) but with potato replacing carrot.
(3) *Plain agar.*
(4) *Oatmeal*: extract of 60 gm. of Quaker oats.

The varieties of cereals used in host reaction studies were: *Triticum vulgare*, vars. Red Fife, Little Joss, Square-head, Reward; *T. monococcum*; *T. dicoccum*; *T. spelta*. *Hordeum vulgare*, var. Plumage Archer.

Inoculum for infecting soil in host reaction studies was prepared by growing the fungus in a mixture of 5 per cent. cornmeal and 95 per cent. soil moistened with water and sterilised at 20 lb. pressure for 1 hour. Inoculum of this type gives excellent growth of the fungus and subsequently heavy infection; it can be distributed evenly in the soil; and it dispenses with the unsatisfactory practice of adding excessive quantities of organic matter to the soil.

(*b*) *Effect of temperature on growth*

The effect of temperatures from 12 to 36° C. on the rate of linear growth on carrot dextrose agar is shown in Table II. In this experiment seven isolates were chosen especially for the variation of their sources of origin. The figures in Table II are the averages obtained from five series of plates each of which was inoculated at the centre with a circular slice, 2 mm. in diameter, taken from a 7-day-old culture.

Table II. *Average daily increase in diameters of colonies, in mm.*

Temp. °C.	Isolate						
	4 A	8 A	102 A	108 A	201 A	202 A	205 B
12	0·2	0·1	0	0	2·4	1·0	2·4
16	4·0	4·7	1·9	1·5	6·6	5·3	5·5
20	8·4	10·2	5·7	6·3	8·6	8·7	8·8
25	10·7	11·3	9·9	11·9	9·8	11·2	10·2
30	2·6	0·1	0	0·2	8·6	4·9	3·8
36	0	0	0	0	0	0	0

In all cases the best growth occurred at 25° C. Furthermore, at that temperature there is remarkably little difference between different isolates in linear growth rate. However, there are marked differences in the range of temperatures over which the isolates are able to grow, some growing with rapidity at both high and low temperatures (*e.g.* isolate 201 A) at which others remained at rest. The English isolates show a wider range for growth than the Canadian ones.

(*c*) *Cultural characteristics*

Six Petri dishes of carrot dextrose agar were inoculated with young growing cultures of each of eight isolates, and were incubated at 25° C.

The tabulated description in Table III was made after 25 days. The "twisting of hyphae" referred to is seen without the aid of a lens and is associated with the formation of strands by the close association of groups of single hyphae. The macrohyphae are those described by Fellows (4). It is to these that the coloration of the colonies is due and not to any marked coloration of the medium. Their importance will become evident later on in this work. It is seen that in these respects the isolates vary greatly on carrot dextrose agar.

Table III

Appearance of cultures after 25 *days on carrot dextrose agar at* 25° C.

Isolate No.	Aerial mycelium	Twisting of hyphae	Macrohyphae
4 A	Dense	Slight	Few
8 A	Negligible	None	Many
102 A	Long and slender	Slight	Moderate number
108 A	Negligible	Strong	None
201 A	Negligible	None	Very few
202 A	Sparse and uneven	Strong	Moderate number
205 B	Negligible	Slight	Very many
302	Negligible	Slight	Moderate number

They were also grown on plain agar, on which all isolates failed to produce macrohypha, although spreading fairly rapidly over the surface of the agar and forming a very meagre mycelium. The production of these macrohyphae is therefore dependent upon the substrate on which the fungus is grown, as well as on the inherent nature of the strain concerned.

(*d*) *Saltation*

The mutability of isolates for various characteristics has been observed by a number of workers, and especially by Russel (11), but the actual occurrence of saltations *in situ* has not been reported.

In November 1934 a culture of isolate 108 A on carrot dextrose agar was found to have some small raised lumps of aerial mycelium, none more than 2 mm. in diameter, scattered in various parts of the dish. These were taken with a fine wire needle, the surrounding agar containing hyphae being first removed. These erumpent masses were placed to grow on potato dextrose agar, and at the same time normal hyphae were transferred to other tubes. By the time the fungus had reached the edge of the tubes in the case of the normal hyphae, those from the erumpent masses had only just commenced visible growth. From then on, however, they rapidly covered the surface of the agar. There was considerable difference in the appearance of the colonies; those from normal hyphae were quite white; those from the erumpent masses were very dark, thick masses of black strands being produced mainly at the point of contact of the glass and the agar. They were found to remain constant for at least four subcultures. The new isolate is pathogenic.

(e) *Survival of desiccation*

Ability to survive unfavourable conditions of the environment must naturally play an important part in the life of an organism like *O. graminis*. Desiccation is probably of special importance in many wheat-growing countries such as Australia and Canada, where summer droughts are common in which the top soil may become very dry. At a time when many workers are concentrating on the question of the survival of this fungus in soil, some knowledge of the reaction of various strains is of value. Furthermore, it has been shown that the isolates used in this study vary greatly in their production of macrohyphae in agar cultures, and the possibility was entertained that they might enable the fungus to survive desiccation.

Two experiments were conducted. In the first, six isolates were used for comparison, three of which produced macrohyphae fairly abundantly on potato dextrose agar (Nos. 4, 6 and 7) and three of which failed to produce them (Nos. 2, 3 and 5). Petri dish cultures on potato dextrose agar were cut into 2-mm. squares to the full depth of the agar (1–2 mm.). These pieces were removed to glass slides and placed in a desiccator over calcium chloride. They were allowed to remain there for periods of 8, 14 and 22 days. At the end of each period ten pieces from each isolate were removed to dishes of potato dextrose agar, which were placed at 25° C. to determine the number of pieces still viable. During the drying process the pieces of agar had become shrunken and so dried on to the glass slides that they could only be removed by allowing them to soak in sterile water, in which manner they regained their normal condition. The number of pieces showing growth were recorded at frequent intervals.

In the second experiment 60 per cent. sulphuric acid was used for drying eight different isolates grown on carrot dextrose agar. The cultures were dried by suspending them over the acid in large covered glass dishes. At various times a culture of each isolate was moistened and ten pieces from various portions were placed on potato dextrose agar.

The number of portions of cultures showing growth, out of ten pieces plated from each isolate, are shown in Tables IV and V.

In both experiments the isolates varied appreciably in ability to withstand desiccation. There was correlation between the presence of

Table IV

Viability of cultures of Ophiobolus graminis *dried over calcium chloride*

No. of days drying	Isolate	Presence (+) or absence (−) of macrohyphae	No. of pieces growing after		
			6 days	13 days	19 days
8	4	+	4	8	—
	6	+	2	7	—
	7	+	3	7	—
	2	−	0	0	—
	3	−	0	3	—
	5	−	0	0	—
14	4	+	0	0	1
	6	+	0	0	1
	7	+	1	2	4
	2	−	0	0	1
	3	−	0	0	0
	5	−	0	0	0
22	4	+	0	0	0
	6	+	0	0	0
	7	+	0	0	1
	2	−	0	0	0
	3	−	0	0	0
	5	−	0	0	0

Table V

Viability of cultures of Ophiobolus graminis *dried over 60 per cent. sulphuric acid*

No. of days drying	Isolate	Macrohyphae	No. of pieces growing after		
			4 days	12 days	30 days
2	4 A	Very few	10	—	—
	8 A	Many	10	—	—
	102 A	Very few	10	—	—
	108 A	Few	10	—	—
	201 A	None	10	—	—
	202 A	Many	10	—	—
	205 B	Very many	10	—	—
	302	Many	10	—	—
6	4 A	Very few	10	10	10
	8 A	Many	10	10	10
	102 A	Very few	0	1	4
	108 A	Few	5	6	6
	201 A	None	0	0	0
	202 A	Many	0	0	0
	205 B	Very many	10	10	10
	302	Many	0	0	4
15	4 A	Very few	0	0	0
	8 A	Many	10	10	10
	102 A	Very few	0	0	0
	108 A	Few	0	0	0
	201 A	None	0	0	0
	202 A	Many	0	1	2
	205 B	Very many	10	10	10
	302	Many	0	10	10

macrohyphae and ability to withstand desiccation, the only exception being No. 202 A, which, though showing many macrohyphae, was rather susceptible to injury. There was no relation between resistance and the source of origin of the isolate.

(*f*) *Differential host reaction*

Extensive experiments carried out by Russel and experiments on a smaller scale by Van der Laar have so far failed to demonstrate resistance to *O. graminis* by wheat varieties, and even by species of *Triticum* other than *T. vulgare*. For the three experiments described here small degrees of infection of the plants were aimed at. Several isolates of *Ophiobolus graminis* were used, since it seemed advisable to obtain a maximum variability of both host and parasite. It is only by doing this that we can hope to obtain any evidence of that small degree of resistance which might be used as a starting-point for the plant breeder.

The method used for all three experiments was fundamentally the same. The fungus was grown for 17 days in cornmeal soil. Similar soil containing no fungus was used for the controls. Flower pots, size $5\frac{1}{2}$ in., were two-thirds filled with sandy loam (Slough) soil sterilised for 2 hours at 20 lb. pressure and were then left for 1 day. On the day that the pots were partly filled the soil inoculum was removed from the flasks and air-dried, and on the following day 5 gm. were scattered evenly over the surface of the partly filled pots. On top of this inoculum were distributed twenty-five seeds of grain, and these were covered with sterilised soil and watered. Five pots were used for each treatment. The pots were kept at room temperature, varying from 10 to 16° C.

When the control plants of the largest varieties were about 250 mm. long, all the plants were removed and measured to the nearest mm., from the base of the stem to the tip of the longest leaf.

For statistical treatment plants from all five replicates were bulked. Standard errors of plant lengths were calculated according to the method of Fisher (5). The "injury index" is the value given by the formula

$$\text{Injury index} = \left(1 - \frac{\text{Length of infected plants}}{\text{Length of controls}}\right) \times 100.$$

That is to say, the injury index is the percentage difference in height between the infected plants and the controls. This injury index is therefore a difference, so that

$$\text{S.E. of injury index} \\ = \left[\sqrt{(\text{S.E. of controls})^2 + (\text{S.E. of infected plants})^2}\right] \times \frac{100}{\text{length of controls}}.$$

Experimental combinations:

Exp. 1. *Ophiobolus graminis* isolates 4 A, 102 A, 201 A; differential hosts *Triticum vulgare*, vars. Red Fife, Little Joss, Reward, Squarehead, and *Hordeum vulgare*, var. Plumage Archer.

Exp. 2. *Ophiobolus graminis* isolates 201 A, 202 A, 205 B, 4 A, 108 A, 102 A, 8 A; differential hosts *Triticum vulgare*, vars. Red Fife and Little Joss, *T. monococcum*, *T. dicoccum*, *T. spelta*.

Exp. 3. *Ophiobolus graminis* isolates 205 B, 302; differential hosts as in Exp. 2.

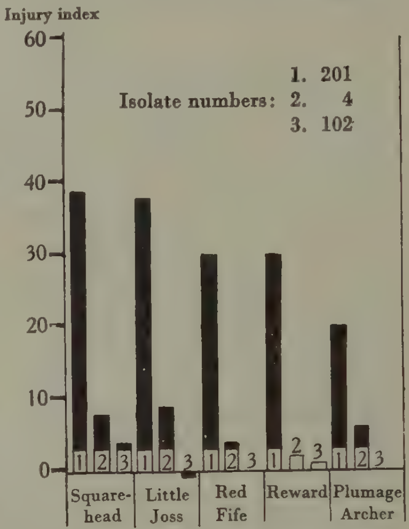

Fig. 1. Injury indices (percentage reduction in length) of differential hosts attacked by *Ophiobolus graminis* (Exp. 1).

The results of Exp. 1 are shown in Table VI and Fig. 1, and those of Exps. 2 and 3 are combined in Table VII and graphically represented in Fig. 2. When these tables are examined as regards the injury indices, either from left to right across the columns, or from top to bottom down the columns, it is at once seen that the injury indices fall steadily with almost unbroken regularity. Where they fall out of order the derangement is slight and usually not mathematically significant. We may conclude, therefore, that the isolates causing most severe injury to one of the hosts will also cause most severe injury to the others. The converse is also true, that the hosts which suffer least injury from one isolate will also suffer least injury from other isolates. This is the reverse of physiologic specialisation.

Table VI

Injury indices of Ophiobolus graminis *on differential hosts—Exp.* 1

Isolate No.	Squarehead	Little Joss	Red Fife	Reward	Plumage Archer
201 A	39·1 ± 2·5	37·8 ± 1·9	29·5 ± 2·5	30·0 ± 2·0	20·5 ± 1·8
4 A	8·2 ± 2·4	9·3 ± 1·6	4·5 ± 1·7	2·3 ± 1·3	5·5 ± 2·3
102 A	3·7 ± 1·8	− 1·1 ± 1·1	0·5 ± 1·4	1·4 ± 1·7	0·5 ± 2·1

Infection ratings, based upon the amount of discoloration produced, used by various workers (3), were also made, but the degree of differentiation was not easy to ascertain, although the results agreed in a general way with the injury indices. It was evident, however, that it was possible to get a marked discoloration of root and stem tissue and yet little reduction in the length of the seedlings. On the other hand, *Hordeum vulgare* showed no discoloration of tissue, although isolate 4 caused significant reduction in length of the plants.

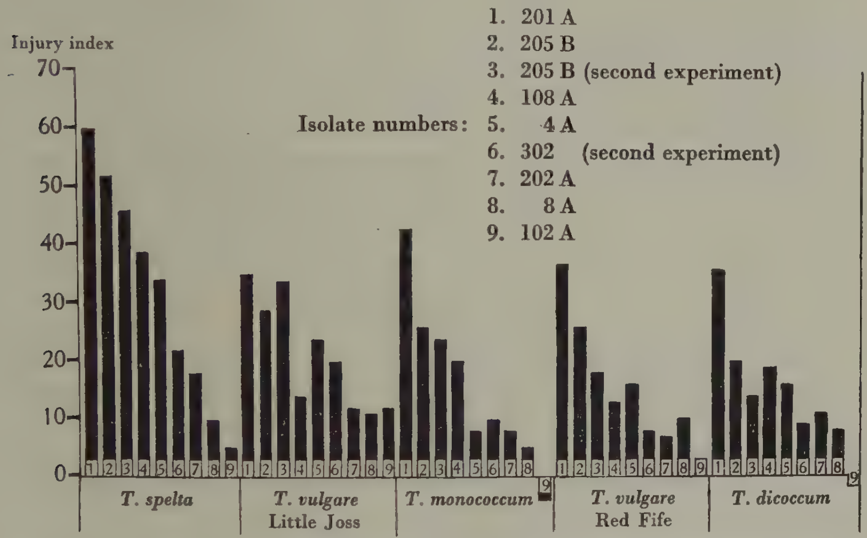

Fig. 2. Injury indices (percentage reduction in length) of differential hosts attacked by *Ophiobolus graminis* (Exps. 2 and 3).

Table VII

Injury indices of Ophiobolus graminis *on differential hosts—Exps.* 2 *and* 3

Isolate No.	*T. spelta*	*T. vulgare*, var. Little Joss	*T. monococcum*	*T. vulgare*, var. Red Fife	*T. dicoccum*
201 A	60·0 ± 4·5	35·3 ± 1·7	42·7 ± 2·4	37·4 ± 2·1	36·2 ± 3·0
205 B	51·7 ± 5·3	28·6 ± 2·0	25·5 ± 2·6	26·2 ± 2·1	20·4 ± 2·8
205 B*	46·3 ± 2·9	33·5 ± 1·6	24·0 ± 2·1	17·7 ± 2·0	14·2 ± 2·6
108 A	38·7 ± 5·6	14·3 ± 1·8	20·5 ± 2·0	12·8 ± 1·6	18·9 ± 2·7
4 A	33·5 ± 5·3	23·7 ± 1·7	7·5 ± 2·0	16·4 ± 1·8	15·8 ± 2·8
302*	22·0 ± 5·1	19·5 ± 1·9	10·0 ± 2·0	7·9 ± 2·2	8·7 ± 2·7
202 A	18·3 ± 5·7	12·0 ± 1·6	7·5 ± 2·0	6·7 ± 1·6	11·2 ± 2·9
8 A	9·6 ± 5·2	11·2 ± 1·6	5·0 ± 2·1	9·7 ± 1·7	7·7 ± 2·9
102 A	5·2 ± 5·3	12·2 ± 1·5	−4·5 ± 1·9	2·6 ± 1·7	−2·0 ± 2·3

* Data from Exp. 3.

Müller-Kögler (8) criticises the use of secondary indices, such as infection ratings on a colour basis, in determining the degree of infection of plants. He points out that the base of the stem of barley is not blackened even after 2½ months in unsterilised soil, although infection is severe as shown by microscopical examination. He recommends as a method of diagnosis the presence of dark hyphae on the surface of the roots, in their epidermal cells, and in the intercellular spaces of the outer cortex, and also microhyphae in the central cylinder and in the vessels. Unfortunately it is impossible to adcpt these methods on a scale sufficiently extensive for the purpose in question, and a secondary index such as the injury index used here probably gives a composite picture of the total effects of the invasion of the various portions of tissue. In the circumstances it is a more useful criterion than colour description. It would be well to reserve these infection ratings for experiments in which varying conditions are compared with one single variety, and not to introduce them in a comparison of different varieties.

III. Discussion

The cultures of *Ophiobolus graminis* obtained from various sources have been referred to as "isolates". The terminology of fine divisions in fungi has been considered by Brierley(2), so that there is no need to discuss it here. No matter how they arose, whether as "continuous" or "discontinuous" variations, the isolates described here exhibit differences sufficient to warrant the use of the term "strains".

The conditions influencing the survival of *O. graminis* in the soil are at the present time being widely studied. Russel(11) found that *O. graminis* survived in unsterilised soil for at least 2 years and then caused severe injury to wheat; Padwick(10), in experiments using a similar technique and a similar type of soil, found that it had become almost innocuous in 2 months. Such differences may well be explained on the basis of strain differences, as indicated by the experiments on temperature curves and desiccation.

Aamodt(1), who has discussed physiologic specialisation from the plant breeder's point of view, points out that the existence of a large number of strains of a pathogenic fungus need not be regarded as rendering the task for the plant breeder impossible, since in many cases they may be divided into a few groups from the point of view of pathogenicity. In fact, the occurrence of marked specificity, of which the best-known example is in *Puccinia graminis*, may be considered somewhat encouraging, since it is evident that there is no strain of the fungus to which some wheat variety is not resistant. Thus there is a place for the plant breeder to start operations. When dealing with less specialised parasites it may well be a difficult problem to find a starting place.

The experiences of several workers have suggested that it might be impossible to discover varieties of common wheat resistant to *Ophiobolus graminis*, and even species of *Triticum* other than *T. vulgare* have proved unpromising (11). This may be in part due to the exceptionally heavy inoculations commonly given, and in part to the manner in which data were taken. Thus in some instances attention was paid mainly to the number of plants attacked in pots of soil heavily infested with vigorous cultures, and usually all the plants were attacked. The experiments recorded here indicate that there is a degree of variation in injury suffered, at least in the seedling stage. None of the varieties used, however, offer any marked degree of resistance.

Although the strains studied varied widely in their pathogenicity, no specificity was exhibited towards any of the hosts used. The host range, although small, was widely divergent in characteristics. High susceptibility towards one strain was accompanied by high susceptibility towards the others, which is the reverse of what holds with many obligate parasites, notably *Puccinia graminis*.

IV. SUMMARY

1. Isolates of *Ophiobolus graminis* compared were obtained in England, Canada and Australia from the following hosts: *Triticum vulgare, Agropyron tenerum, A. repens* and *Hordeum murinum*.

2. The optimum for growth of all isolates studied on carrot dextrose agar was in the neighbourhood of 25° C. They varied in their ability to grow at temperatures above and below the optimum.

3. Isolates vary in type of growth on carrot dextrose agar, the most noticeable difference being in the production of macrohyphae.

4. Saltation leading to the abundant production of black strands of macrohyphae by a culture producing very few was observed.

5. There are distinct differences in ability to survive desiccation over calcium chloride or 60 per cent. sulphuric acid. There seems to be some relation between the presence of macrohyphae and ability to withstand desiccation.

6. Isolates exhibit marked differences in general pathogenicity, but these differences are not specific towards certain hosts. Those causing greatest reduction in length of plants of *Triticum spelta* also caused greatest injury to *T. dicoccum, T. monococcum, T. vulgare* varieties and

Hordeum vulgare. Conversely, hosts most resistant to one isolate were also those most resistant to the others.

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TULIP FIRE

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INTRODUCTION

THE tulip crop is one of increasing importance, and it is stated of one variety alone (William Copeland) that at least 20,000,000 are grown annually (2). Any factor, therefore, that can be so devastating as to make the bloom from the crop of little or no value is one worthy of fuller investigation. Such a factor is the organism that causes the fire disease. The present writers have studied various aspects of this disease in different localities, and they have co-operated in expressing the results of their findings so that a clearer view may be taken and result in a more efficient control of the disease in the forcing house and field.

NAMES

(*a*) *Disease*

The name "tulip fire" is generally familiar to growers, and with a few exceptions is the name used by them. Fire is the same as the Dutch name of the disease "vuur", and it is probable that the English name was adopted from this. Prof. Westerdijk informs the writers that the first use of this name in Holland is uncertain. In England it is referred to by Jacob in 1912 (26).

Other names of the disease are tulip mould (32, 11), *Botrytis* disease and *Botrytis* blight (22). None of these seems as good as tulip fire, and this is the name adopted by the British Mycological Society (7). The name "spot" is also sometimes used for certain phases of the disease; some growers imagine that this is a disease distinct from fire, but this is not the case.

(*b*) *Fungus*

The name adopted by the British Mycological Society is *Botrytis Tulipae* (Lib.) Lind (7), replacing *Sclerotium Tulipae* Libert (1830) and *Botrytis parasitica* Cavara (1888), and also *B. Tulipae* (Lib.) Hopkins. The writers consulted the Plant Pathological Laboratory, Harpenden, on the grounds for this latter replacement. From them it is learned that the combination was first made in 1913 among the errata to Lind's *Danish Fungi as represented in the Herbarium of E. Rostrup* (p. 650), as it was discovered that the name *B. parasitica* had been used by Fries for the fungus now known as *Peronospora parasitica*. The specific name was apparently derived by Lind from the *Sclerotium Tulipae* of Madame Libert. Klebahn (29) took exception to any such derivation of the specific name on the ground that it was no longer possible to prove that the sclerotia named *S. Tulipae* are indeed those of the "fire" fungus by obtaining the *Botrytis* from them.

On this point the writers obtained the opinion of Miss E. M. Wakefield, The Herbarium, Royal Botanic Gardens, Kew. She states, *in lit.*: "The question whether you should cite Lib. in brackets for the combination *Botrytis Tulipae* is a matter of taxonomic opinion, namely whether *Sclerotium Tulipae* Lib. is actually the same fungus as the *Botrytis*. Personally I think there is no doubt. I have examined the Kew example of Crypt. Ard. No. 36, and can see no difference of structure between these sclerotia and dried sclerotia of *B. Tulipae*. Klebahn (*Jb. Hamb. wiss. Anst.* XXIV, 3 Beiheft, 1907, p. 5) says that he examined the specimen at Berlin and that 'in appearance it corresponds exactly with the sclerotium of *Botrytis parasitica*, only the hyphae in the sclerotium are slightly less thick and have a somewhat more swollen wall'....Also he admits (29) that *Scl. Tulipae* Lib. is probably the sclerotium of the 'fire' fungus....Further, Cavara quoted *Scl. Tulipae* as a synonym when he was describing *B. parasitica*, and Lind does so also in the body of his work. Therefore I think it is fair to assume that when Lind changed the name to *B. Tulipae* in his errata he had Madame Libert's specific epithet in his mind and was using it in his new combination."

DISTRIBUTION

Tulip fire is to be found wherever tulips are grown. Early records are Belgium 1830, Italy 1888, Holland about 1885, Norway 1890, Germany 1902 (22). It has also been recorded in Denmark (45) and Russia (22).

In England the first published reference probably is that made in 1889 (43), and some years later in 1897 (6) specimens were sent to the Royal Horticultural Society. Massee was familiar with it in 1899 (32), and Carruthers reported its occurrence in 1900 in Herefordshire (9).

As there were so few early English records that mentioned this disease it was thought of some interest to examine herbarium specimens of tulips and to see if any of them showed "spots" that might be attributed to this fungus. In the Herbarium at the Botany School, Cambridge, specimens were examined from two collections of *Tulipa sylvestris* L., and in both cases on certain specimens a few fleckings were observed, and these appeared similar to the spots that are caused by the fire fungus. In one case the specimen was from Forfar, dated 1847, and was from Babington's collection. The other was from the collection of T. Martyn, Professor of Botany at Cambridge University, 1761–1825. The specimen was dated 1762 and probably came from the Botanic Gardens. Although this matter cannot be pursued in further detail, it is thought of interest to record, since it seemed unlikely that this crop, that has for so long a period aroused the interest of amateur and professional growers, had escaped attack.

Since the inception of the reorganised Advisory Service (about 1922) the disease has been recorded every year in many counties (1, 3, 4). It is common also in Scotland (5) and Ireland. In America the first record, 1901, is by Halsted (19) in New Jersey. Numerous later records (12, 13, 20, 25, 30, 31, 39) indicate that the disease is common throughout Canada and the United States.

ECONOMIC IMPORTANCE

Fire is the most serious disease that attacks the tulip crop, and the annual losses are large and particularly so in certain seasons, but statistics that deal with these are not available, and it would serve no useful purpose here to quote the financial losses of individual growers. It is sufficient to say that in a bad attack, in the forcing house or field, the bloom is unmarketable and valueless. The direct loss to the bulb crop on account of diseased bulbs is small even when the flowers are spotted, but the indirect loss is by no means negligible in crops intended for bulb production, where the flowers are "topped". A substantial proportion of the increase in size and weight of new bulbs already formed is made during the period subsequent to "topping". Early withering of the foliage caused by the disease is sufficient to reduce the number of bulbs attaining the larger and more valuable grades. Heavy spotting is enough to induce such premature withering without the development of fire lesions.

Severe losses by fire were reported in England in 1924 (1), in Holland on Murillo tulips in 1925 (37), and in Canada (25) and U.S.A. (31). It was bad also in Canada in 1927 (12, 13), and 1928 and 1930 were bad seasons in south-west England (3). In west Cornwall in 1927 and 1928, fire was so severe that several growers were unable to market their bloom. In the latter year the disease was also bad in East Anglia. In 1929 the season was a good one in both Provinces, and although many blooms were spotted they realised a good price. It should be stated here that spotting does not always make a crop unsaleable; it is when the petals are blistered that the disease makes the bloom definitely unmarketable.

In the forcing house total loss is a common penalty for the smallest neglect, as care and experience in preparing the boxes and subsequent management of the crop is essential if fire is to be avoided.

HOST SPECIALISATION

Botrytis Tulipae is found only on tulips. Other species of *Botrytis* are found on many other plants and are not distinguishable from *B. Tulipae* except in artificial culture. This fact probably explains reports of the occurrence of tulip fire on other hosts, *e.g.* on hyacinths, narcissi and crocuses in Denmark(45) and of *B. Paeoniae* passing from lily of the valley on to tulips under glass in Germany(16).

B. galanthina on snowdrops is very similar to *B. Tulipae*, and Smith thought it was the same fungus(43). Massee knew that the fungi were distinct and that cross-inoculations were unsuccessful(33).

Hopkins(22) inoculated lily of the valley, onion, lily, narcissus, hyacinth, crocus, gladiolus, paeony, potato and golden seal with *B. Tulipae* and failed to obtain infections except with injured leaves and stems of narcissus and crocus. On these two hosts inoculations were successful with mycelium, but not with conidia.

As far as is known the tulip is not susceptible to other species of *Botrytis*. A large number of cultures made by the writers from the lesions on primary infections have given uniformly *B. Tulipae*. It may be, however, that other species are capable under certain conditions of causing spots on the leaves and petals. The only aberrant culture was from one taken from Farncombe Sanders in pots in Devon in 1931: this was a strain of *B. cinerea*.

One of us (A. B.) attempted to inoculate tulips with the following species of *Botrytis*: *B. elliptica* from lilies (1931), *B. narcissicola* from narcissi (1931 and 1934), *B. Paeoniae* from lily of the valley (1935), but these were negative. *B. narcissicola* also failed to infect tulips in each season; in 1931 sclerotia were used, and in 1934 conidia on detached narcissus leaves.

SUSCEPTIBILITY OF VARIETIES

Although there does not appear to be any inherent resistance of any tulip variety to *B. Tulipae* when subjected to infection under favourable conditions, nevertheless there are considerable differences in the readiness with which beds of different varieties go down before an epidemic. Whether this is due to differences in inherent susceptibility, to accidental differences such as later time of appearance of the shoots or flowers, to predisposition to mechanical injury and consequently—if the inoculum is present—to the production of large spore-bearing lesions, or to physiologic specialisation by particular strains of the organism, remains to be established. For these reasons it might be misleading to attempt any classification of varieties on a basis of degrees of susceptibility.

In Devon and Cornwall Baronne de la Tocnaye is the variety least often spoiled by fire, and Hopkins (22) remarks that this variety was the only one to stand out clean in a garden in 1917. A well-known grower in East Anglia expresses the view that the May-flowering *Gesneriana* varieties such as Gesneriana Major and Gesneriana Lutea, the May-flowering variety Retroflexa and all the varieties which have *Gesneriana* blood in them are more susceptible to fire than any others. These varieties are also very susceptible in Devon and Cornwall. Among the more widely grown Darwin varieties, William Pitt, William Copeland and Bartigon stand out as being the most troublesome in nearly all districts: in East Anglia William Pitt is considered by the majority of growers to be the most susceptible of this group.

SYMPTOMS

Three sets of disease symptoms may be produced by *B. Tulipae* which may be called fire, spot and a rot of the bulb. The circumstances leading to their appearance will be discussed later; at present only the symptoms will be described.

(*a*) *Fire*

The typical fire lesions are grey or greenish grey, variable in size and shape, and somewhat sunken compared with the surrounding healthy tissues (Plate II, fig. 1). The lesions are very common at the tip of the leaf, but may appear on any part of the leaf surface, and often the whole leaf surface is involved, especially if, as frequently happens, several lesions coalesce. There is a definite dark margin to the lesions, which in moist weather can sometimes be split up into three zones, a light green area outside with no spores, then a light grey area with a few spores, and the dark grey or grey-brown zone with abundant spores. These primary lesions enlarge rapidly in moist weather but desiccate in dry weather and often become split and torn by the wind, and the spores shaken away like dust. The fire symptoms may appear quite early in the season, for example in January in forcing boxes before they are moved into the greenhouse. In the field they may be seen shortly after the shoot has speared through (Plate II, fig. 2). In East Anglia in 1935 they were apparent in the third week in February, and the percentage infection noted at this date in a field of William Pitt was 0·6. At this stage slugs not infrequently injure the tissue and so create an ideal medium for subsequent infection. If the lesion is formed before the young leaf has opened out the latter will be malformed, while in some cases the young shoot is so badly attacked that the plant fails to grow. Such plants are easily missed when the beds are inspected and may serve as the inoculum to infect the field. Fire lesions may arise on the green stalk as well as on the leaves and resemble the leaf lesions in every respect (Plate II, fig. 3). They are very common at the base of the stalk and in this position are easily overlooked. Sclerotia form readily on the decayed flower stalks, but are rarely seen on decayed leaves in the field. As the decay proceeds the leaves wither and fall to the ground.

The number of conidia produced during the season must be considerable. When a drop of water impinges on a spore-bearing lesion the minute radiating splashes a millimetre or less in diameter may contain large numbers of spores. In one such splash 1 mm. in radius over 100 spores were counted. In favourable conditions new spores may be formed at the borders of the lesion in 24 hours.

(*b*) *Spot*

If the plants near to a tulip bearing fire lesions are examined, small spots will probably be found, and these will have arisen from spores that have been blown or splashed by rain from the primary fire lesion. These spots are small, dry, very slightly sunken and yellowish white or greyish white in colour. They are generally circular in shape, and on close examination are seen to have a pale grey centre surrounded by a yellow ring, which is in turn surrounded by a dark green border with indefinite margin. The spots are produced in large numbers, and in showery weather it is difficult to grow a crop of tulips entirely free from them. Individual spots do not become larger unless by coalescence. Theoretically one would expect these spots to develop into primary fire lesions, assuming that the fungus in the spot was alive. If, however, for some reason the fungus died, then, of course, the spot would remain unchanged. To test this ten isolations were made from leaf spots, and in only one of the cultures was there any fungal growth. In another similar series *B. Tulipae* was isolated on one occasion, but it is possible that the spot was recontaminated after its surface was sterilised. Spotted leaves and plants have repeatedly been kept in moist chambers to see if the spots would enlarge. After a week the material begins to turn yellow and die, but if the spots are isolated ones and the material is not bruised or wounded there is no further enlargement of the spots. In some cases mycelium has developed on the spots, and sometimes conidia and pure cultures of *B. Tulipae* have been obtained from these. When the leaves died sclerotia eventually appeared. Plants growing in pots and bearing spotted leaves have been kept in a moist atmosphere under a bell-jar, and still the spots have not changed in character or even formed spores. The bulk of the evidence suggests that the fungus in the spots is dead, but there is some evidence that some of the spots at least may contain viable mycelia. Further observations are evidently required to elucidate the nature of these spots.

Many growers think all spotting is due to hail, but this is not so. Hail spot can be imitated experimentally by dropping small wrinkled peas from a height of 30 or 40 ft. on to tulip plants. Hail spots are light and evenly coloured, generally small in size and very irregular, rarely circular. Under certain conditions hailstorms may accentuate a fire attack by scattering the inoculum and wounding the tissue.

(c) *Rot*

The third type of decay is very different in character from the other two. The whole plant is a sickly pale or yellowish green, under-sized, with flaccid leaves and often no flower, or only a small flower which is not marketable. With a little practice plants can rapidly be picked out by recognising the difference in the shade of green. Fire and spot lesions may be present, but not invariably, and the sickly appearance can be observed earlier than the time when these lesions are formed. The plants can be dug up easily, and with rare exceptions black sclerotia are found on the scales (Plate II, fig. 4). In one case in south Devon in 1925 a 60 per cent. loss of this kind was observed. Many of the fleshy scales may be discoloured or brown and the bulb may rot away early without any new bulbs being formed (Plate III, fig. 1). In extreme forms of the disease small sickly brown shoots grow out covered with sclerotia and die before or soon after reaching the soil surface. Sclerotia may also be carried up on the young leaf-sheath. In these cases it is not always easy to distinguish recently formed sclerotia from those of the preceding season. Bulb rot is much less common than fire or spot and is not described by most writers, the only references we can find being by Westerdijk (46). This form of the disease probably only occurs on the most susceptible varieties, and only when bulb infection is unusually large or the bulbs have been badly bruised.

Such attack on the bulb is also very similar to the disease caused by *Sclerotium perniciosum* and known in Holland under the name of

"Smeul" (42). The sclerotia of this fungus are of the same size and colour as those of *Botrytis Tulipae*, which can only be distinguished from them by cultural methods.

It should be explained here that as a tulip bulb is only a one year's growth the bulbs harvested are usually clean, but in some circumstances, described below, may carry infection.

(*d*) *On flowers*

Fire lesions may occur on petals and on unopened flower-buds in the same way as on leaves. They are grey and bear abundant spores. They spread over the surface very rapidly and soon involve the whole bud or petal. When the bulb is attacked the stalk often remains healthy but does not elongate as the bud does not develop. This phase of the disease is often called "bud blight". These diseased buds form a prolific source of spores.

Spotting of flowers is the commonest symptom and often occurs on an epidemic scale. Two types of flower-spot can be distinguished, which may be termed "spot" (Plate III, fig. 2) and "blister spot" (Plate III, fig. 3).

Spot is similar to the leaf-spot. It is a dry white spot, very slightly sunken, inconspicuous on yellow and white varieties but very striking on red flowers. On some varieties these spots may be very small (*e.g.* minute flecks in Sultan). On the flower, before it is fully coloured, the spots are generally green with a slightly darker centre, and with red or purple varieties the spot shows a dark purple border. The spots become white when the flowers fully open, but otherwise they do not develop or change in any way. The blister spots are white, *puckered and raised*, and they enlarge into a lesion with sharply defined margin even in the dry air of a laboratory or living room. In moister conditions the lesions spread still more and eventually form spores. Economically blister spot is more serious than spot, since affected flowers are not marketable, as the period elapsing during transport is sufficient to allow conspicuous lesions to develop.

Spores form readily on diseased flowers when conditions are favourable, and the fungus spreads to the stamens and stigma, especially if the flowers are diseased before they are fully open, as a moist atmosphere is thus maintained. Generally if spotted flowers are not gathered within a week, lesions with spores will develop on the anthers.

Hail spot damages flowers in a similar way to its effect on the leaves, and the remarks on the latter apply equally well to flowers. The tissues of the petals are very delicate and form a ready prey to *Botrytis* spores. Spots develop in about 24 hours, and in moist conditions the petals decay and sclerotia are formed in abundance in little more than a week. This explains why it is so important to collect and remove decaying tulip flowers in the fields, forcing houses and packing sheds.

(*e*) *On fruit*

Tulip capsules are very susceptible to fire (18). Spot, fire lesions and complete decay are possible exactly as on the leaves and flowers. The chances of tulips setting seed are therefore severely limited by fire, and in south-west England ripe capsules are very rarely observed. Even if the capsule is not attacked the stalk just below it often is, and spores may be formed there. The capsule then fails to form any seed. Capelletti reports finding mycelium of *B. Tulipae* in the seed (8).

(*f*) *On bulbs*

Fire lesions are dark yellow or brown, depressed, with one or more sclerotia; usually rather small (6 mm.) and circular, but in one case observed they were large, grey and irregular with very many sclerotia. (Examined in October after being left in the soil.)

Circular lesions due to *Penicillium* spp. such as *P. corymbiferum* Westl. are very common and should not be confused with fire lesions. They are seldom depressed and are paler in colour and form a green mould when moist. These and certain other fungi, although probably not primary parasites, are often associated with the condition known as chalkiness (Dutch "verkalking", German "steinkrankheiten"). In this condition the bulbs become hard and chalky: the trouble is seen in storage and is due to rough and careless handling of the bulbs.

Bulbs affected with rot show numerous sclerotia on either side of the scale surface, and the scales are often somewhat discoloured at first, then turning brown and finally decaying. Bulbs of this type may decay completely before lifting, leaving sclerotia in the soil. Sclerotia may occur *between* the white bulb scales. They are frequent at the base of the old stalk and inside the old brown bulb scales, and will often be removed when the bulbs are cleaned. Some may be formed inside the new brown scale or tunic and not laid bare except by peeling the bulb.

The proportion of new bulbs affected with one or more of the above symptoms is generally small. Imported consignments have sometimes been returned on account of fire, but the vast majority pass any reasonable inspection. Similarly, bulbs lifted by the grower of a crop in which the plants have been badly attacked can as a rule safely be planted again. The explanation is that the tulip forms entirely new bulbs round the base of the flower-stalk. Fire causes decay of the latter and forms sclerotia on it, but in the process of lifting and grading most of these stalks are rubbed off, so that sclerotia become hard to find on any sample. For example, in 1928 at Newton Abbot, a bed which suffered very badly from fire was lifted and the bulbs numbering about 1000 were carefully examined. Only four bulbs were found with any sclerotia. Hopkins (22), however, records cases of 4·6 and 5·2 per cent. bulb infection with sclerotia present, but he states they were probably lesions from the previous year. In Norfolk the variety William Pitt is markedly susceptible, and in an experimental plot of 10,000 bulbs the attack was so devastating that there was no single bloom marketable in the season 1934. Subsequently in a cursory examination to obtain suitable demonstration specimens, four trays of bulbs of this variety were examined and only seven specimens bearing sclerotia were obtained. Many of these bulbs had developed lesions on which *Penicillium* spp. and *Fusarium* spp. were present. On this same farm the bulbs were carefully harvested and stored in the usual way, but some bulbs were left in the field and these were harrowed up. Subsequently these bulbs were forced in one part of a house, but they started to rot very quickly, and the majority of them on being pulled up at the end of February were bearing sclerotia. It is very necessary that bulbs should be handled carefully and not bruised.

The sclerotia are generally on the decayed base of the flower-stalk, quite small portions of which may remain attached to the bulb. They may also occur on the brown external papery scale, but bulbs with the lesions on the white fleshy scales bearing sclerotia are much less common. It is possible that in an abnormal season, *e.g.* a wet June, infection of the new bulbs occurs forming lesions with sclerotia, and such bulbs next season give rise to the rot of the bulb. At present it may be stated that the problem is not a simple one, as if bulbs bearing sclerotia on the outer surface are planted in pots and kept under observation they may give rise to healthy plants or plants with fire lesions, but no bulb rot has been observed. In one experiment with William Copeland the amount of fire in the shoot was small, but abundant sclerotia were present on the remains of the old bulbs. It may be that rotting of the bulb would have developed in different weather conditions, if the bulbs had been bruised, or if the sclerotia had been more deeply seated. In a larger scale experiment at Kirton, 4018 bulbs with sclerotial material were planted in 1932 and 490 clean bulbs. In 1933 the total number of plants with fire lesions (primary infections) was only six. Extensive spotting arose from these. There was no bulb rot (44). Frequently, as was the case with Copeland just mentioned, there are abundant sclerotia on the husk of the old bulb, the new bulb inside being quite clean. If lifting is not done too early, this outer husk sticks to the soil like a plaster cast, especially if the soil is at all heavy, and the new bulb slips out quite clean. But even when the husk is lifted it usually breaks off as it dries, so that very little remains attached to the new bulb.

The development of bulbs from non-flowering plants is somewhat different and perhaps gives greater scope for the carrying on of infection. The single large leaf has a base which is a white scale completely surrounding the embryo bulb. This white scale eventually withers and becomes the brown tunic of the new bulb, and usually has a long pointed neck where the lamina has broken off. The brown tunic then in this case is a relic of the old bulb, whereas in the new bulbs formed from a flowering plant, the whole bulb including the tunic is new.

LIFE HISTORY

(a) *Conidia*

The conidia appear on the leaves in suitable conditions and may also be formed on all the parts of the flower and on the flower-stalk and the capsule, but never on the bulbs. Their microscopic appearance is similar to the common grey mould, *Botrytis cinerea*, and microscopically the differences are very small. Klebahn (29) places both species in the subgenus *Phymatotrichum* instead of *Polyactis*, and lists the morphological characters of different species. The size he gives for *Botrytis Tulipae* conidia is 10–15 × 6–9 μ, mean 12·4 × 7·6 μ, and the conidiophores are 17–20 μ wide. Hopkins (22) gives 12–24 × 10–20 μ, while Westerdijk (48) gives the majority 15–18 × 8·5–11·5 μ. Saccardo gives 16–20 × 10–13 μ. Our own observations show similar variations, and it is evident that although the spores are generally larger than those of *B. cinerea* (49) the species cannot be recognised by conidia alone. Klebahn (29) considers that many different species including the above should all be included under the species *B. cinerea*.

(i) *Formation of conidia.*

The conidia are formed on the *living host* both under high and low light intensities but less readily in the dark. On detached leaves in a moist dish the fungus continues to spore only while the leaf is alive; afterwards, under suitable conditions, sclerotia are formed and the leaf decomposes.

On cultural media such as Dox and potato agar contained in tubes and kept under normal laboratory conditions spore production is very sparse. It has been increased by submitting cultures to sunlight.

Prolific spore production was obtained by both of the following methods. Tubes of Dox's medium were inoculated with spores in the normal way, and these were then gummed by strips of paper to a window pane so that they would receive a high intensity of sunlight. After 8 days they were sporing profusely. Check cultures kept in the dark had not spored at that time but had commenced to form sclerotia (Plate III, fig. 4).

Abundant conidia were formed when ordinary glass slides were coated with Dox medium, this inoculated with spores, and the slides kept at a high humidity.

On the leaves conidia are not formed if the humidity of the atmosphere is too low, although they can withstand dry conditions for a considerable time. A very high humidity (90–100 per cent.) is necessary for their formation, and a 24-hour period is sufficient at April temperatures. The spores are formed *all over the old lesion*, especially on the under side.

(ii) *Dispersal of conidia.*

The conidia are very easily detached and are dispersed by wind and splashed off the lesions by rain. In an average plantation the spores are not in sufficient numbers to contaminate the air to any marked degree. The spores blown from the primary infections are scattered in a few seconds over a large area, and in 2 or 3 min. may be a great distance away, so that the spore load of such wind currents would be relatively very low. The air in the immediate vicinity of a fire lesion is well charged with spores and the plants surrounding it are bombarded with them; secondary infections are rarely observed more than 20 or 30 ft. from the point of origin of a primary infection.

The spores adhere readily when blown on to the wax-like surface of a tulip leaf and they are not easily dislodged.

The conidia may be spread by the splashes arising from raindrops. Experiments showed that a drop of water falling from a height of 13 ft. on to a diseased leaf scattered spores over an area of at least 3 sq. yards. Even for this small area a field with 1·3 per cent. fire lesions more or less uniformly scattered over it would be completely spotted, assuming there were about 120,000 bulbs per acre. The minute splashes that result from impact of raindrops on these fire lesions are highly charged with spores.

In one small splash covering approximately 3 sq. mm. 156 spores were counted. As a high humidity is necessary for germination, and as these spores germinate quickly, it is clear that this method of dispersal is particularly favourable for infection.

In the forcing house a fertile source of infection results from the condensation of water on the glass, the dropping of this on to fire lesions, and consequently the scattering of the spores.

It has been observed also that aphis may transfer the fire spores from plant to plant in the greenhouse.

(iii) *Germination of conidia.*

The conidia germinate very readily in hanging drop cultures, and the rapidity with which spots arise in the field shows that the spores must start germinating almost immediately after alighting on the surface of the leaf. Germination in hanging drops is markedly stimulated if a minute piece of a tulip leaf is placed in the drop. It is well known that exosmosis of nutrient substances takes place from leaves in nature, so that this will encourage more rapid germination. This is also confirmed by the fact that germination in dewdrops collected from tulip leaves is more vigorous than in ordinary tap water. This stimulus to germination in the hanging drop is the same whether a piece of healthy green leaf is added or a brown decayed portion. On artificial media such as potato agar and Dox and when under high humidity the conidia germinate under any intensity of visible light and also in the absence of it, but when exposed directly to the lower wave-lengths of ultra-violet light from an artificial light source they are killed, the time period depending on the distance from the source of light.

When germinating spores are exposed on synthetic media to strong visible light from which injurious ultra-violet light has been excluded by filters, the growth of the germ tube and later the mycelium is inhibited in varying degrees and the lateral growth retarded. The hyphal strands have a gnarled appearance and the growth is more localised than when such cultures are submitted to low visible light intensities. When spores are germinated under low light intensities such as would exist in a laboratory, the growth is the accepted normal one. At room temperature (64° F.) the spores start germinating very quickly, and after 2 hours germ tubes are observed, after 3 hours approximately 20 per cent. have germinated, and so progressively until the maximum is reached. The development under suitable humidity and temperature conditions is then rapid.

Hanging drop cultures form a simple method of studying the effect of poisons on spore germination. A minute trace of copper sulphate will inhibit germination, whether tulip leaf-tissue is present or not, but little or no inhibition takes place with the relatively insoluble copper carbonate.

The effect of fungicidal dusts on germination can be well studied by means of glass slides with an agar surface. Spores are gently blown on to this, and then the fungicidal dust is dropped from a suitable height and the slide moved through the drift. In this way an even deposit is obtained. The slides are then kept at high humidity and periodically examined. Employing this technique it was found that laboratory copper carbonate dust and flowers of sulphur did not materially affect the germination of the spores. They germinated in intimate contact with such particles after a period of 2 hours, and subsequent development to a period of 4 days afterwards appeared normal. In the above cases the mycelia grew over the dust deposits and formed spores. Germination did not take place when copper-lime dust was substituted for copper carbonate dust.

(iv) *Infection by conidia.*

This aspect has not been studied. According to Hopkins (22) *B. Tulipae* does not form an appressorium but penetrates directly either through stomata or between epidermal cells, and he figures both inter- and intracellular mycelium.

(v) *Viability of conidia.*

The same writer (22) reports that conidia kept dry in the laboratory germinated after 13 and 30 days, but not after 51 days. One of the present writers obtained 100 per cent. germination after storage for 30 days, and germination was vigorous at 1–50 days and then declined considerably, but weak germination of a few conidia was still possible after 6 months. It would seem, therefore, that if the sources of the inoculum—the fire lesions—were removed at the earliest date possible, the risk of subsequent infection would be minimised.

(*b*) *Sclerotia*

The continued growth of the mycelium involves the subsequent formation of sclerotia. In the favourable environment of a moist chamber they begin to appear on the living shoot or in artificial culture in from 9 to 12 days. On the same medium killed by steam sterilisation the period is longer, about a month; on leaves they have been observed after

16 days. In the greenhouse and in the open sclerotia appear on the leaves, stalks and bulbs at varying periods. They are not invariably formed, but badly diseased shoots infected early usually show large numbers. The rapidity of formation is probably greatest in a greenhouse or late in the season. For example, tulip plants potted in contaminated soil on April 21, 1931, developed lesions on which sclerotia began to form in a fortnight; and leaves already bearing fire lesions that were covered with soil and kept in the greenhouse developed sclerotia in a week. Somewhat similarly inoculated plants in the greenhouse formed severe fire lesions in a week, and when lifted a fortnight later sclerotia had formed on the lower part of the stalk and at the neck of the bulb. The evidence of these and similar experiments suggests that sclerotia are formed approximately between 10 days to 3 weeks, generally within a fortnight, the time depending on the suitability of the environmental conditions. In the open field the formation of sclerotia is generally less rapid. In 1935 in East Anglia they were noted on the stalks as early as March 6, and also on decomposing leaves covered by clods of earth. They were found in quantity in Devon and Cornwall in the early part of April 1933, and also in East Anglia at this period in 1934 and 1935.

In dry environments sclerotia may not be formed and the flower-stalks dry up normally.

(i) *Germination of sclerotia.*

Artificial cultures of *B. Tulipae* are easily made by sterilising and washing the surface of sclerotia, cutting them up with a sterile knife, and placing the sections on agar or other medium. Germination seldom takes place if undamaged sclerotia are used. *A priori*, however, one would assume that the primary infections of healthy plants arise from mycelium formed by the germination of sclerotia in the soil. The following experiment shows that such germination does take place. In Devon on April 14, 1931, sclerotia were sterilised, washed, and poured on to damp soil in large dishes. No germination was observed, and in July the soil was stored in a cupboard. It became dry in the summer, but sterile water was added in October. On February 29, 1932, there was still no change, and the dishes were transferred to a cold shed. On April 1 a very small amount of mycelium was observed from which three cultures were made on April 13. All developed typical *B. Tulipae*. By the end of April the mycelium on the dishes was abundant and characteristic. This experiment indicates how tulip fire may start afresh each spring from sclerotia in the soil.

(ii) *Viability of sclerotia.*

How long can tulip fire sclerotia remain alive in the soil? Field observations have shown that 2 years is too small an interval between successive plantings, the disease reappearing on epidemic scale in spite of the ground being kept very clean and free from tulips in the interval. This would indicate that the sclerotia can live over 2 years. Hopkins (22) regards them as longer lived, and states that successful isolations have been made from sclerotia several years old. Dutch opinion, however, is in favour of 2 years as the period of viability (38). In this investigation sclerotia from laboratory cultures were viable after 1 year, but as yet we have no complete evidence as to the germination of older ones.

Inoculation experiments in Devon have shown that sclerotia less than 1 year old readily produce the disease, but the few experiments that have been carried out with older sclerotia have given negative results.

A staining method is available by which it is possible to determine quickly whether sclerotia are alive or dead. Living tissues do not stain well with eosin and it washes out completely in a few hours, whereas dead tissues take up the stain more readily and it does not wash out even in 12 hours (*R.A.M.* VIII, 1929, p. 263).

The resistance to chemicals is of great importance for the control of tulip fire, and the following data have been arrived at by the above methods:

Copper compounds. 2 per cent. copper sulphate solution is innocuous: the sclerotia can be left in it for several days without harm.

Mercury compounds. Sclerotia did not germinate after 2 hours in 0·05 per cent. mercuric chloride solution though the control (in tap water) grew well. They germinated if the period of immersion was from 5 to 15 min.

Formalin. After 15 min. in undiluted formalin, only two pieces out of sixteen grew while controls gave 100 per cent. germination.

Hot water. Five minutes in boiling water killed sclerotia. It is proposed to try the effect of periods of immersion in the hot-water bath used for daffodil bulbs.

The effect of mercuric compounds was studied also in a different way. In another investigation that was being made at Cambridge on the toxicity of various organic and inorganic compounds of mercury it was found that certain of the organic type were very strong fungicides at very weak dilutions. Sclerotia were steeped for varying periods in certain of these and then cultured. Using methyl mercuric chloride at a dilution of 1:100,000 the sclerotia were killed after immersion for 2 hours but not after immersion for 1 hour. Five hundred William Pitt tulip bulbs treated in the same way were uninjured, and 500 treated with a stronger concentration of 1:50,000 were similarly not affected. It is intended to carry out further investigations with this material.

GROWTH IN ARTIFICIAL CULTURE

The fungus is readily cultured on a wide range of artificial media forming many black sclerotia, the size and number increasing with the richness of the medium used. On Edson's acid agar only white mycelium is formed, no sclerotia. Aerial spores under normal conditions of laboratory culture are rarely formed, but profuse sporulation is obtained if cultures are submitted to high light intensities. Similar cultures under low light intensities produce sclerotia. Hopkins (22) records that conidial formation can also be induced by partial drying.

On sterilised tulip tissue spores are formed under low and high light intensities. Good growth is also obtained on sterilised snowdrop leaves, but on narcissus stalks it is poor. On sterilised tulip bulb tissue the fungus grows moderately well and forms sclerotia.

ARTIFICIAL INFECTION

(*a*) *By conidia*

If uninjured tulip plants are dusted with spores by means of a camel-hair brush and then sprayed with sterile water from an atomiser, spots will generally appear within 24 hours if a high humidity is maintained. The perianth segments are particularly susceptible, decay is rapid and sclerotia are sometimes formed within a week.

Artificial epidemics may be initiated by placing diseased leaves bearing spores on healthy plants, and natural epidemics by omitting to rogue out the primary infections. In both cases the secondary symptoms are the same, an intense spotting. If tulip tissue is wounded and inoculated with spores, decay quickly sets in and a lesion bearing copious spores results. This, in effect, constitutes a primary fire lesion and initiates spotting of the plants in the immediate neighbourhood. By artificial infection and by observation of natural infection in the field it has seemed clear that the intensity of an epidemic depends upon the quantity of the inoculum (*i.e.* the fire lesions).

(b) *By sclerotia*

Experiments carried out in Devon and Cornwall both in the greenhouse and the field have shown that tulip fire can be induced by placing sclerotia at the time of planting near uninjured tulip bulbs. Infection has been produced in this way when sclerotia have been taken from cultural media and also from naturally infected bulb scales. In one case the sclerotia were 18 months old. Infection in the above experiments was by mycelium arising from the germination of sclerotia, but mycelium from artificial cultures can be used with equal success.

(c) *By diseased soil*

It is recorded that the disease can be contracted from contaminated soil, *i.e.* soil containing sclerotia rising from the remains of a previous tulip crop (10, 14, 18, 25, 38, 39, 40, 44). This is the general experience in East Anglia and the south-west. Mr W. F. Cheal, the Horticultural Superintendent for the Isle of Ely *in lit.*, says that tulips grown on the same land for 2 years will undoubtedly develop fire, and Mr H. Goude, the Horticultural Superintendent for Norfolk, supplies similar information, and this also is our own experience. In the south-west it is always observed that old beds are badly attacked, whereas the newly planted ones are less so. For example, in 1927 a farmer near Exeter planted £200 worth of bulbs which gave him a handsome profit. He left the bulbs in the ground and next season (1929) did not pick a single flower. A similar mistake was made by another grower in east Devon. Other growers in the district who lifted their bulbs and planted in fresh ground were able to market all their blooms. Detailed experiments at Seale-Hayne College confirm the general field experience. Bulbs have been planted in soils that have grown tulips in previous years, and the resulting infections have been in sharp contrast to appropriate check plots where tulips have been grown in soil that has not previously grown them. In experiments of this nature the tulips on the virgin land have shown fewer primary infections, less spotting and a much greater increase in the weight of the bulb crop. Somewhat similar experiments have been made by artificially contaminating soil in the field with sclerotia, and comparing with non-inoculated soil. Fire developed more severely on the contaminated soil.

It would seem therefore that both in the south-west and East Anglia soils can become "sick", and that this "sickness" is due to the contamination of the soil by sclerotia.

EPIDEMICS AND THE WEATHER

It is well known that many fungus disease epidemics can be correlated with meteorological conditions, and tulip fire provides one of the best examples of this. Rainfall, humidity and temperature are the three important factors. Rainfall is important in providing the requisite conditions for germination and infection. It also spreads the spores by radiating splashes. As it is preceded by high humidity, which is maintained while rain is falling, spore formation is encouraged. The strong winds, which often accompany or follow the rain in the spring, still further spread the spores. Temperature is probably less important, as the fungus thrives at low temperatures and spores are not formed above 25° C. (34). At higher temperatures the low humidity usually prevailing is the limiting factor.

In Devon and Cornwall a study has been made of the effects of rainfall and humidity on the development of fire. In the past 10 years (1924–34) there has been a close correlation between the rainfall in the months of March and April and fire epidemics. When the rainfall has been high in these months there has generally been a severe epidemic. The relationship, however, is not exact. For example, in 1932 and 1933 fire was slight, although the rainfall was as great as in 1930 and 1931, when fire was severe. The reason for this was that the humidity in 1932 was much less than in 1931. The weather conditions in 1931 were cold and wet and unsettled, but in 1932 they were mainly anti-cyclonic. Anti-cyclones were also dominant in 1933, and the humidity was particularly low in the first 3 weeks of April.

Similarly in other districts high rainfall encourages fire epidemics, and a cold wet spring is generally regarded as favouring it. Although the fungus flourishes at lower temperatures than is usual among fungi, it is doubtful whether temperature is ever a limiting factor in England, as conditions are normally cool in March and April. The temperature may be high enough in May to inhibit fire, but the low humidity that usually accompanies high temperatures is probably the deciding factor.

There is close correspondence between the progress of an epidemic and the relative humidity; if this is high and is maintained, the attack is intensified.

CONTROL MEASURES

The control of this disease is bound up with the proper cultivation of the crop. At the outset then it is possible to distinguish between certain groups of cultural considerations which become of importance or not according as to whether the plant is being grown for flower production or primarily for the production of marketable bulbs.

(a) *Flower production*

(i) *Lifting and transplanting.*

This is an important method of controlling tulip fire and is necessary if good quality bloom is to be produced with reasonable certainty. Although in small cottage gardens tulips are sometimes grown for many years without getting fire, this is because in a small bed there is less chance of infection arising. On a field scale, however, unless they were to be periodically rogued, and very carefully, it would be unwise to leave the bulbs down. The safest procedure is to lift all the plants and to put into fresh soil.

(ii) *Deep planting.*

It is claimed that if tulips are planted deeply at 8 or 12 in. they can be left down for several years and relied upon to suffer very little from fire. The reason suggested for this is that the primary infections are reduced in number. Experiments carried out in Norfolk in 1933 on the variety William Copeland gave the following results. Five hundred bulbs were planted at 4 in. deep and a similar number at 8 in. The percentage primary and secondary infections were as follows:

	Planted 4 in. deep %	Planted 8 in. deep %
Primary infection	1·6	1·4
Secondary infection: early April	76	46
early May	100	97

A somewhat similar experiment was made with variety William Pitt in 1934. A plot consisted of four rows, each containing 118 bulbs, and these were randomised and replicated four times. One series was planted at 4 in. and another at 12 in. The percentages of primary infection and bulb rot were as follows:

	Planted 4 in. deep %	Planted 12 in. deep %
Primary infection	1·0	0·6
Bulb rot	0·3	0·6
Total infection	1·3	1·2

It would seem therefore that there is a correlation between the depth of planting and the percentage of primary infection and bulb rot. At Gulval in Cornwall results do show a much reduced amount of primary infection when the bulbs are planted at 12 in., but the percentage of bulb rot has not been determined, and it may well be that the percentage of fire lesions plus the percentage of bulb rot would be approximately the same whether planted at 4 or 12 in. deep. The disadvantage of such a method is that it involves additional time and labour, as it is difficult to lift the bulbs.

That it is not impossible for tulip fire infection to reside at a great depth is shown by an observation made in the early part of May 1934 on a sandy soil in Somerset where tulips had been planted 12 in. deep. Some of these plants were attacked by fire, and when one bulb was carefully removed sclerotia were found on the brown sheath just below the soil surface and also on the bulb 12 in. down. Similar observations were made in Norfolk in the early part of March 1935, and sclerotia were found on the bulbs and stems of tulips that were planted 12 in. deep.

(iii) *Late planting.*

An experiment was carried out at Seale-Hayne College in which several rows of Pride of Haarlem bulbs were planted in diseased soil on November 3 and December 1, 1930. The bloom was a week late on the later sown bulbs, but the majority of the flowers were marketable, but none were saleable from the early planted. There was no significant difference in the weights of bulbs lifted. Although in west Cornwall late planting has occasionally been practised with success, as a rule planting conditions are too bad to make this method feasible on a large scale.

(iv) *Spacing.*

The flower growers' practice of planting bulbs rather closely together in order to get the maximum number of flowers per acre is very favourable to tulip fire, because there is a greater chance of spores alighting on leaves if the plants are close together; also the humidity amongst such plants is maintained at a higher level as there is less circulation of air. Moreover, close planting makes it less easy to observe the fire lesions, and also increases the risk of mechanical injury to other plants when these are removed. Experiments have shown that wounded tissue makes an ideal medium for the germination of fire spores, and such infected tissue gives rise to fire lesions.

Planting in rows is therefore better than in beds, and the wider the pathways the better, as there is then less risk of leaves being trampled. Gibson (15) stresses the importance of planting a good distance apart, and we have seen many cases of its beneficial effect.

(*b*) *Bulb production*

It has been previously stated that the tulip forms a complete new bulb each season: the bulb-grower lifts his crop each year, cleans off the old scales to obtain his saleable product, and returns the stock to land which has been free from tulips for at least 2 years. If the land has been badly contaminated by sclerotia a longer period is necessary.

Deep planting and late planting experiments have been carried out at Kirton during the past 4 years under the direction of Mr D. E. Horton (23, 24), and they have shown that early planting (September) and shallow planting (not greater than 5 in. in the case of the commoner varieties) are alike essential if the bulb-grower is to obtain the increase and the proportion of larger bulbs and the good quality that he desires. These primary considerations far outweigh any value which the opposite practices may possess in the direction of "fire" control: on the other hand, the bulb raiser is bound to indulge in fairly wide spacing to achieve the best results: in this direction cultural requirements and those of disease control are the same. Apart from the above, the more important issues in the control of this disease apply alike whether the plant is being grown for the flower or the bulb crop.

(i) *Cleaning bulbs.*

In 1929–30 experiments were made in the south-west to determine if it was profitable to clean the bulbs, *i.e.* to remove the old scales and base of the old flower-stalk. The tulips planted were 500 Farncombe Sanders and 500 William Copeland from Penzance, where they had been badly attacked by fire in 1929.

There was no significant difference between cleaned and non-cleaned, as the crop in both cases was remarkably healthy and vigorous. In this season, therefore, there was no advantage in cleaning the bulbs. It is possible, however, that in more favourable seasons such as 1928 this cleaning operation would have decreased the number of primary infections. Unfortunately we have no data for other years.

(ii) *Liming and manuring.*

It is often stated that liming is beneficial (13, 17). There appears to be little evidence for this and we have seen fire bad on soils well provided with lime, nor can we find any relation between epidemics and soil acidity and lime content.

The avoidance of nitrogenous manure is another common recommendation (36). In order to test this an experiment was carried out at Seale-

Hayne College in 1931–2. Nine beds of William Copeland and Pride of Haarlem (alternating) were planted in ground which had not grown tulips before. The site of beds 1–4 had been heavily manured with dung dug in during October 1931; the remainder received no manure. There was 2 per cent. of fire in beds 1 and 3 (William Copeland), but none in beds 2 and 4 (Pride of Haarlem) or in the unmanured beds of these two varieties. The beds were left down a second year as they were in a sheltered position. A fair amount of fire developed, and its variations showed no relation to the manuring. It would seem, therefore, that, if the soil has not grown tulips before, the ground may then safely be manured in preparation for tulips. This is a common practice in bulb cultivation and the results are satisfactory.

(iii) *Bulb disinfectants.*

Formalin. In both the south-west and East Anglia work on bulb disinfection has been carried out with this fungicide. In Devon experiments were made in 1928, 1929 and 1933, and bulbs were dipped in formalin at concentrations of 1:160 and 1:320.

In East Anglia a product containing formalin was used in 1933.

The varieties that have been treated in such experiments were Farncombe Sanders, William Copeland, Pride of Haarlem and William Pitt.

Bulbs so treated have been planted in clean soil and contaminated soil, and compared with bulbs that have not been treated, but there was no effect on the growth, flowering, or the amount of fire.

It has previously been shown that the sclerotia were to some extent able to survive immersion in strong formalin.

Copper sulphate. 2 per cent. copper sulphate solution is recommended by some writers (14). The following experiments do not support its use, and it has been shown above that sclerotia are not harmed by this treatment. In 1928 Pride of Haarlem was treated with 2 per cent. copper sulphate (20 min.) and 4 per cent. copper sulphate (dipped). The experiment was repeated in 1929. In both cases the amount of fire was not reduced. Bulbs treated in this way have been planted in clean and contaminated soil, but there has been no control of fire.

Mercury compounds. Experiments on the dipping of bulbs in inorganic or organic mercury compounds using either laboratory chemicals or proprietary products have been carried out in the south-west and East Anglia, and the bulbs have been planted on clean and contaminated soil. In the south-west in 1929 and at Kirton in 1933 (44) no favourable results were obtained.

In 1932–4 various inorganic or organic mercury compounds were used either in plots at Cambridge or at a farm in Norfolk. Bulbs prior to planting were either dipped in the solution, dusted with fungicidal dusts, or wrapped in papers impregnated with the solutions. No satisfactory results were obtained.

In 1934, 500 William Pitt bulbs *soon after lifting* were steeped in a solution of methyl mercuric chloride at a concentration of 1:100,000 for 2 hours, 500 were steeped for a similar period in water, and 500 were left unsteeped, and they were planted in clean soil. Less fire lesions were noted in those steeped in the fungicidal solution. Further experiments are in progress, and if these should be satisfactory they will be reported at a later date. At present no recommendation can be made.

(iv) *Other treatments.*

At Seale-Hayne College in 1929 other treatments have been used, and these included steeping in water at 45–50° C. (the temperature falling from 45 to 34° C.) and also treating in dry heat at about 50° C. The variety was Pride of Haarlem, and the bulbs were planted in diseased soil. The bulbs were not injured, but no control of fire was obtained. In 1930 William Copeland bulbs of all sizes were immersed in the hot-water bath used for the control of daffodil eelworm. The bath was maintained at 110° F. (43·5° C.) for periods of ½ hour, 1 hour, and 1½ hours. The bulbs immersed for the longer periods were all killed and only 5 per cent. survived the ½-hour treatment, and five of these subsequently showed primary lesions.

In the work on bulb disinfection that has been made by the writers the treatments with fungicidal solutions with one exception have been made immediately prior to the planting of the bulbs. Work is now in progress at one centre on the treatment of the bulb when it is lifted.

Hall (18) recommends dusting bulbs with sulphur, but we have not carried out experiments to test this.

(v) *Soil disinfection.*

We have no data on the use of chemicals. Van Slogteren (41) suggests the use of steam. The results from carbolineum seem to be doubtful (27, 49) or harmful (38).

(vi) *Spraying and dusting.*

Results in this direction obtained by the writers have not been very promising. The difficulties are that tulip foliage is very susceptible to injury from copper sprays, *e.g.* Bordeaux Mixture (22, 39) and Burgundy

Mixture. Spray injury or dust injury is most undesirable because the injured tissue forms a breeding ground for the fungus, and, in effect, acts as a primary source of infection. The second difficulty is that the tulip leaf is not easy to wet and the spray runs off in drops. Unless a spreading agent is used it is impossible to obtain an adequate cover, and consequently such spraying must be ineffective. The third difficulty is that the fire lesions are continually liberating their spores and are bombarding the plants in their immediate neighbourhood. Theoretically, therefore, to obtain control of the disease it would be necessary to have a permanent fungicidal cover on the leaves, and this would necessitate a large number of applications of the particular fungicide. In Norfolk in 1934 various products were used as sprays or dusts. These contained either copper compounds or sulphur. The Saanichton potash sulphur-resin spray was also used (35). Duplicated plots each containing 500 William Pitt bulbs were used in the experiments, and one, two or three applications of the particular spray or dust were given and these were made on the following dates: March 21, April 5, and April 16.

The primary fire lesions were not rogued from these plots, so that the foliage was continually being bombarded with spores and liable to infection.

The various treatments employed all gave a fair to moderate measure of control in the initial stages, but it would serve no useful purpose here to give the relative percentage infection, since when the applications were discontinued, and *when the conditions for the development of the fungus became more favourable, then fire became rampant.* At harvest there was no difference between any of the treated plots or between these and the untreated plots, as all alike had a maximum infection and the bloom was unmarketable. It may be that if the particular treatments had been continued to a later date a measure of control would have been obtained.

There is a variation in the opinions that are expressed by growers on the subject of applying protective fungicides to this crop.

The present writers, as the result of observations and experiments that they have made in Devon, Lincolnshire, and Norfolk, are of the opinion that if beds are not periodically examined and the fire lesions removed, then one, two or three spray or dust applications with sulphur or copper compounds will not result in a marked measure of control.

If the primary fire lesions are removed it is thought that dusting or spraying the crop may further reduce the risk of spotting and also any extensive development of fire lesions that may arise through the infection of wounds that have arisen through mechanical damage, but there is no conclusive evidence on this point. It may be, however, that strict attention to careful roguing would render any subsequent operation of this nature unnecessary. It should be stated here that any spray or dust operation that is carried out should be such that the materials used cause no scorch damage, because if this takes place the scorched areas become suitable media for the development of the fire fungus and consequently spread the disease. Copper compounds in particular if incorrectly used may cause such damage. Any spray or dust material that may be employed should be used as directed by the particular manufacturer, and a preliminary experiment should first be made.

(vii) *Shelter.*

In the south-west it has been found that the provision of adequate shelter minimises fire; this should not be so close, however, that high humidity is encouraged. The shelter should break the force of the wind but not eliminate it. Some of the worst cases of tulip fire in Devon and Cornwall occur in small gardens which are completely shut in. Shelters made of canvas or coir netting or laths bound together are widely used in the south-west for both tulips and daffodils, and they cannot be dispensed with. There is little evidence that cold winds do more harm than any other wind, though this is often implied (10). On the contrary, cold winds in the spring are often associated with dry weather, which is not favourable to fire. On the other hand, the lesions caused by frost lead ultimately to infection with *Botrytis*. The use of other flowers, *e.g.* wallflowers or narcissus, as a shelter is of doubtful value; the beneficial effect with tulips is probably one of spacing rather than shelter.

(viii) *Avoidance of mechanical damage.*

Mechanical damage whether due to wind or human agency or other accidental causes would appear to account for a great proportion of fire lesions not arising directly from diseased bulbs or contaminated soil. It cannot be too strongly stressed that disease will result if tulip tissue is bruised or wounded in any way. Not only does this apply to the flower and foliage but also to the bulb. In one particular instance in a Norfolk house the bulbs had been badly bruised; this initiated a severe rot of the bulbs and subsequently a fire attack developed. It has been shown (23) that tulip bulbs which have suffered mechanical injury during the storage period are not only liable to suffer from certain types of storage rot, but also give rise to plants more prone to fire attack. Three years' experiments at Kirton have shown this very clearly. Bruising insufficient to show any visible damage is enough to cause it, and in the best practice any bulbs accidentally dropped on the floor are discarded. Mechanical damage to the foliage can be greatly lessened if wider paths are left between the beds; these can be made by leaving a second blank furrow.

The bloom also should be handled with the greatest care so that mechanical injury does not take place, otherwise fire may develop after they have been dispatched to market. For example, the variety Bartigon, forced in a greenhouse under excellent conditions of low relative humidity, can be so damaged by inexpert bunching that large areas of the outer leaves become bruised and water-soaked. This provides at the same time a very favourable substratum for the growth of the fungus and ensures a high relative humidity in the box, and this may result in fire development during transit.

It is suggested that a useful purpose would be served if farm operatives were informed of the nature of the fire disease and told how this could largely be circumvented by attention to the roguing of fire lesions and the prevention of all needless mechanical injury.

(ix) *Roguing of primary infections.*

Fire is an excellent example of a disease that can be brought under control by strict attention to hygienic details. The foremost of these is of paramount importance. *It is the roguing of primary infections* (*i.e. fire lesions*). Whatever method of cultivation is adopted this measure in the opinion of the writers is essential. The fields should be inspected periodically from February onwards, and those plants showing fire lesions should be removed bodily and placed in some suitable container so that the spores already present on them are not scattered. In this operation great care must be taken by operators to see that they do not cause mechanical damage to neighbouring plants. Subsequently this diseased material should be burnt and not dumped on to a refuse heap. In showery weather an inspection and roguing should be made once a week.

On one farm on which experiments and observations have been made in East Anglia the cost of a first roguing was 10*s*. an acre. When practising no method of control the same grower estimated his losses in a bad attack of fire as from 50 to 100 per cent. in the value of the flowers and a negligible increase in stock.

It is of marked importance that tulip tissue, whether healthy or diseased, should not be left lying about in the fields, houses, or packing sheds, as otherwise fire lesions may develop upon it. These in effect are identical to the primary infections that have been rogued from the fields. In the packing sheds after a day's work there should be no debris left lying about. It should be cleared up and burnt.

It is stated that the roguing of primary infections has been carried out in Holland regularly for many years with complete success (38).

(c) *Discussion on control measures*

The writers are of the opinion that successful fire control will be attained if bulbs are handled carefully so that mechanical damage is avoided, lifted each year, cleaned, and planted in fresh soil and the primary infections rogued, destroyed, and care taken to avoid unnecessary wounding of the tissue. Subsequent operations such as spraying or dusting must be considered as subsidiary adjuncts in control; and, if used, care must be taken to avoid scorch damage. Under the conditions of their own experiments the writers have not obtained results of much promise, but further experiments in this direction and also on the disinfection of bulbs immediately after harvest are in progress.

SUMMARY

The salient points that deal with the disease of tulip fire are discussed, its names, the various forms of the disease such as fire, spot and a rot of the bulb, the symptoms of these, the distribution of tulip fire, its economic importance, its host specialisation and the relative susceptibility of varieties to it.

The life history of the organism causing it, *Botrytis Tulipae* (Lib.) Lind is given, and the formation, germination, viability, and development of conidia and sclerotia in natural and artificial media are discussed. Infection studies in the laboratory and field with conidia and sclerotia are described, and the meteorological factors that favour artificial or natural epidemics.

Observations and experiments dealing with control measures are given.

Concluding this study the writers wish to acknowledge with sincere thanks the assistance given by growers and those otherwise interested in the cultivation of this crop, particularly to those that have given land, material, and the benefit of their experience.

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EXPLANATION OF PLATES II AND III

PLATE II

Fig. 1. Fire lesion on a leaf of William Pitt.
Fig. 2. A primary infection. Variety William Pitt.
Fig. 3. Fire lesion on a stem, showing zoning.
Fig. 4. Bulb rot of William Pitt, showing sclerotia.

PLATE III

Fig. 1. Bulb rot of the variety William Pitt.
Fig. 2. Spot symptoms.
Fig. 3. Blister spot symptoms.
Fig. 4. Cultures of *Botrytis Tulipae* grown in sunlight and darkness.

(*Received June* 22, 1935)

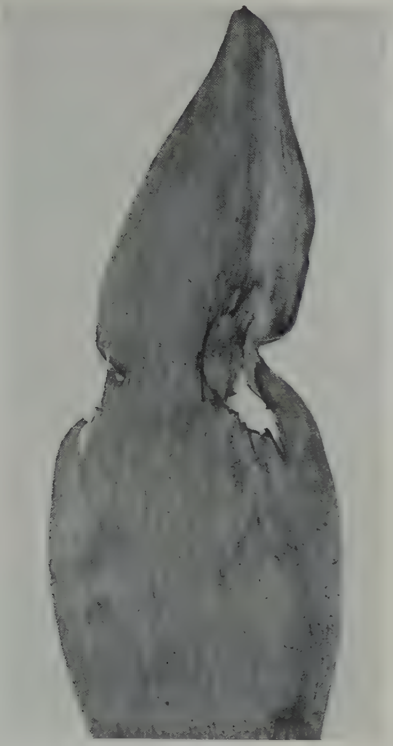

Fig. 1.

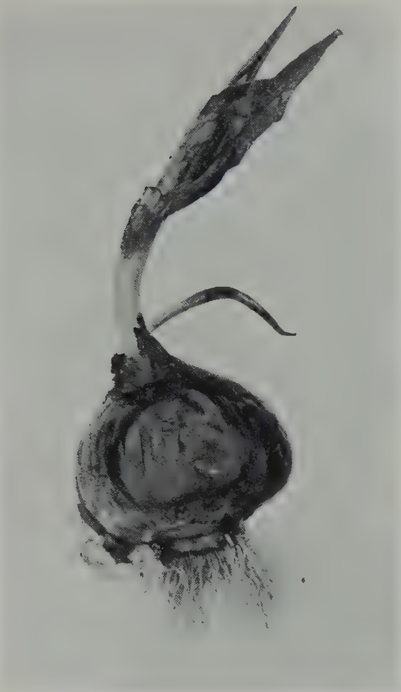

Fig. 2.

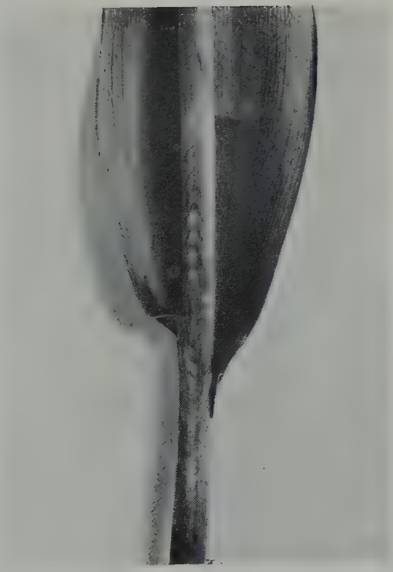

Fig. 3.

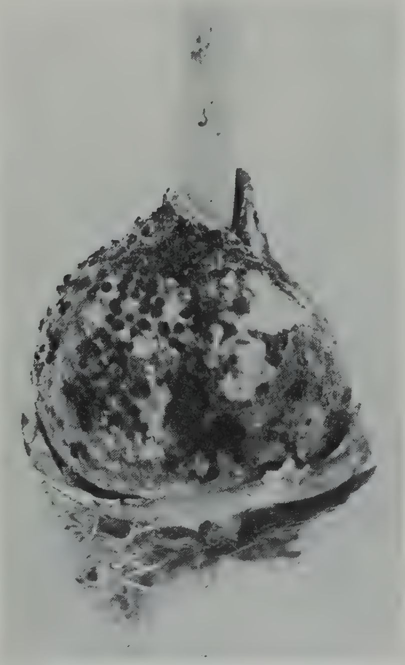

Fig. 4.

BEAUMONT, DILLON WESTON AND WALLACE.—TULIP FIRE (pp. 57–88).

SOME EFFECTS OF TOBACCO MOSAIC VIRUS ON THE GROWTH OF SEEDLING TOMATO PLANTS

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(With 5 Text-figures)

Introduction

The symptoms shown by tomato plants when infected with the virus of ordinary tobacco mosaic (*tobacco virus* 1, J. Johnson) are well known. In summer the disease is characterised by a light green-dark green mottling and slight distortion of the leaves, but in winter the mottle is absent, leaf distortion is more severe, and there is a development of anthocyanin in the stem. All observers agree that some stunting of the plant occurs and that there is a decrease in crop. Very little quantitative data has, however, been published on either of these effects, although an account of experiments investigating the decrease in plant size and crop due to mosaic for both field and greenhouse-grown tomatoes has been given by Heuberger and Norton (4), who briefly review the earlier work.

The aim of the work described below was to obtain quantitative data of the effect of tobacco mosaic virus on the growth of seedling tomato plants. Parallel series of healthy and infected plants were grown at different times throughout a complete year, and the growth rate of each series was measured by determining the dry weights of the plants from samples taken at convenient intervals.

Methods

Ninety to one hundred similar tomato seedlings, var. E.S. 1, growing in 3½-in. pots and having two or three foliage leaves, were divided at random into two groups. One group was inoculated with a standard strain of *tobacco virus* 1 by lightly rubbing the terminal leaflets of the first two foliage leaves with a piece of muslin soaked with infected juice, and the other group was similarly treated with distilled water. The two

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Table

Summary

Sample ...		1					2				3			
Series	Date	T.	L.		Dry wt.	W.C.	T.	L.	Dry wt.	W.C.	T.	L.	Dry wt.	W.C.
	1934													
I	Apr. 13	1	3–4	C.	0·054	92·60	7	5–6	0·22	91·68	12	7–8	0·53	91·72
	Apr. 30			I.	0·052	92·49			0·19	91·55			0·40	90·62
II	May 24	0	3	C.	0·051	91·22	8	6	0·26	90·70	14	7–8	0·70	91·66
	June 11			I.	0·047	91·34			0·27	90·18			0·52	90·82
III	June 25	0	3	C.	0·043	91·85	7	5	0·34	91·43	11	6–7	0·80	91·27
	July 16			I.	0·049	91·85			0·34	91·26			0·89	91·00
IV	Aug. 22	1	2–3	C.	0·034	91·70	12	6	0·38	91·92	16	7	0·78	91·83
	Oct. 3			I.	0·034	91·90			0·34	91·72			0·67	91·28
V	Oct. 22	0	2	C.	0·020	91·96	18	4–5	0·12	92·06	25	6–7	0·18	92·46
	Jan. 9			I.	0·020	91·94			0·10	91·92			0·15	91·61
VI	Nov. 5	0	2	C.	0·027	91·59	21	4–5	0·10	94·41	31	7–8	0·14	94·49
	Dec. 17			I.	0·034	91·69			0·11	94·17			0·15	93·94
VII	Dec. 4	0	3–4	C.	0·013	94·37	30	7–8	0·07	95·10	52	11–12 (10)	0·25	93·19
	Jan. 25			I.					0·05	94·19			0·16	92·44
	1935													
VIII	Jan. 17	0	2	C.	0·005	94·48	18	4	0·021	93·30	28	5–6	0·06	93·29
	Mar. 31			I.					0·021	92·37			0·04	92·35
IX	Mar. 4	0	2	C.	0·013	92·88	16	4–5	0·104	91·95	22	6–7	0·35	90·79
	Apr. 16			I.					0·102	91·74			0·32	90·04

T. = time in days after inoculation.
L. = leaf stage. (Numbers in brackets = leaf stage of infected plants when different from controls.)
C. = control. I. = infected. W.C. = percentage water content. Dry wt. = mean dry weight of ten plants.

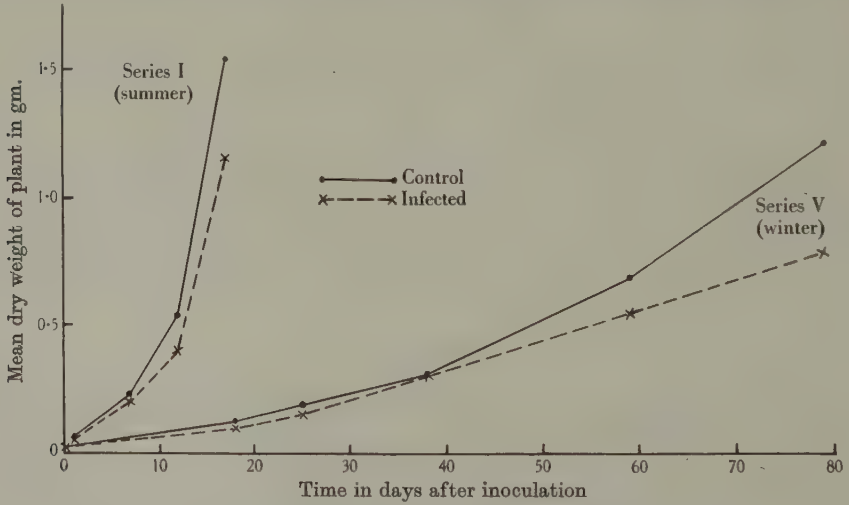

Fig. 1. Typical summer and winter growth curves.

groups were grown under the same conditions in an insect-free greenhouse. The average temperature of the greenhouse was 69° F. in summer and 58° F. in winter.

results

4				5				6				7			
.	L.	Dry wt.	W.C.	T.	L.	Dry wt.	W.C.	T.	L.	Dry wt.	W.C.	T.	L.	Dry wt.	W.C.
7	9–10	1·53 1·15	91·40 90·61	—	—	—	—	—	—	—	—	—	—	—	—
9	9–10	1·52 1·24	92·20 91·64	—	—	—	—	—	—	—	—	—	—	—	—
1	10–11	3·20 2·60	— —	—	—	—	—	—	—	—	—	—	—	—	—
1	13	10·9* 7·5*	90·96 91·74	—	—	—	—	—	—	—	—	—	—	—	—
8	8	0·31 0·30	94·08 93·38	59	11–12 (10)	0·68* 0·55*	93·18 92·49	79	15–16 (13–14)	1·33* 0·91*	93·15 92·98	—	—	—	—
2	9–10	0·24 0·22	94·04 93·06	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
9	9 (7–8)	0·21 0·13	92·77 91·99	48	9–10 (9)	0·54 0·37	92·21 92·20	56	11–12 10–11	1·20* 0·67*	90·57 90·58	63	13 (11–13)	2·78* 1·57*	91·42 91·17
28	8–9	0·75 0·70	90·83 90·49	43	11–12	4·36 3·15	90·63 90·76	—	—	—	—	—	—	—	—

* Means of five plants.

At the beginning of an experiment, immediately after inoculation, ten plants were taken at random from each group, and the fresh and dry weights of stem, leaf and root determined. Weighing was carried out on spring balances and the material was dried to constant weight in an oven at 70° C. under reduced pressure (methods described by Bolas and Melville (1)).

A second sample was taken at the time that visible symptoms appeared, when the plants were at approximately the five-leaf stage. Further samples were taken at intervals up to the ten-leaf stage, beyond which the plants were too large to be handled in samples of ten, but, in three experiments plants were transferred to 6-in. pots and grown to the fourteen-leaf stage, when samples of five plants only from each group were weighed.

RESULTS

Nine series of experiments were carried out over a period of 12 months and the results are summarised in Table I. Fig. 1 shows growth curves for typical summer and winter series (series I and V), mean dry weights of whole plants being plotted against time in days after inoculation. These curves show clearly the lower growth rate of infected plants and also the low growth rate of both healthy and infected plants in the winter.

The growth curves have been examined in two parts: (1) the parts representing growth before the appearance of visible symptoms, *i.e.* growth during the incubation period, and (2) the parts representing growth after the appearance of visible symptoms when virus is present in all parts of the plant.

The data were subjected to a statistical analysis, but owing to the unexpectedly high variance of the material, the differences between healthy and diseased plants in one experiment are not usually significant. Larger samples could not be taken owing to limitations of apparatus and material.

Incubation period and growth rate

The incubation period may be defined as the time between inoculation and the appearance of visible symptoms. During this interval the virus multiplies at the point of inoculation, then spreads throughout the plant, initiating a growth response. That the incubation period bears some relation to the growth rate of the host plant has long been known, for symptoms appear 7 days after inoculation in summer and after 3 weeks in winter, but no attempt appears to have been made to establish such a correlation.

In these experiments a negative correlation, having a correlation coefficient of 0·947 (p less than 0·01), was shown to exist. Fig. 2 shows the correlation diagram for incubation period and growth rate. The growth rates were calculated by a formula given in the next section.

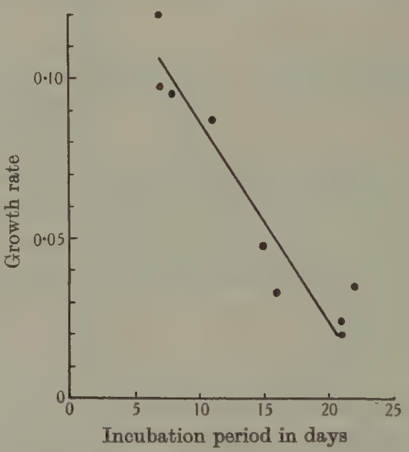

Fig. 2. Correlation diagram for incubation period and growth rate.

Since visible symptoms due to this virus are, in some measure, a growth response (only those leaves developed after infection showing symptoms), such a correlation is only to be expected. It is possible that the time taken for the virus to spread from the point of inoculation to the growing point of the plant is approximately the same summer and winter, and that the longer incubation period in winter is largely accounted for by the slower growth of the plant. Some delay in the spread of the virus in winter, however, seems probable, for according to

Samuel (6) movement of virus from the inoculated leaflet may be delayed in a slowly growing plant.

In the winter of 1931–2 several experiments on the effect of yellow mosaic virus[1] on tomato seedlings were performed, and it was found that a delay in the removal of starch during the night from the leaves of an infected plant occurred a fortnight before visible symptoms appeared. This is evidence that the virus affects the metabolism of the leaf during the incubation period. It is interesting to notice that the starch effect was first observed 7 days after inoculation, which approximates to the incubation period in summer.

Growth rate after the appearance of symptoms

To compare the effect of the virus on the plant at different times of the year the growth rate was calculated over the periods corresponding to the intervals between the appearance of the fifth and the tenth leaves. It was found that over these periods, plotting the logarithms of the dry weights against time gave curves which approximated to straight lines and the slope of these curves was taken as a measure of the growth rate, *i.e.* growth rate $=\frac{\log W_{t_2}-\log W_{t_1}}{t_2-t_1}$, where W_{t_1} and W_{t_2} = the dry weights of the whole plants at t_1 and t_2 days after inoculation, so that K (the efficiency index) $=\frac{\text{growth rate}}{\log_{10}e}$. Fig. 3 shows the growth rates for both healthy and diseased plants plotted against time of year. It will be seen that the mean growth rates of infected plants are invariably lower than the healthy and that the season has a very marked effect on growth. The relative differences between the two sets of growth rates, however, appear to be almost independent of the season. This also has been expressed graphically in Fig. 3 by plotting the growth rates of infected plants as percentages of those of the controls.

Caldwell (2) states that although a tomato plant infected with yellow mosaic virus in spring or summer is considerably smaller than a healthy plant of the same age, it is at the same morphological stage as the healthy plant. In these experiments with ordinary tobacco mosaic virus it was noted that although healthy and diseased plants were always at the same morphological stage in summer, after the ten-leaf stage in winter there was a difference of one or two leaves between healthy and diseased (see Table I).

[1] *Tobacco virus* 6, J. Johnson.

Water content

The mean percentage water content of the plants at every sampling is given in Table I.

On the appearance of symptoms the percentage water content of infected plants invariably fell below that of the controls, and after reaching a minimum, rose again and at a later stage became higher than that of the controls (see series IV and IX). The actual differences in percentage water content between the diseased and healthy plants for the nine series are expressed graphically in Fig. 4. The observation that infected plants may have a higher water content than healthy is in agreement with the findings of other workers. Heuberger and Norton (4) found the water content of plants 15 in. in height, inoculated with tomato mosaic virus at the five-leaf stage, to be 91·86 per cent., the controls having a water content of 91·61 per cent.; and in a second experiment with larger plants, that the values were 91·94 and 91·59 per cent. for diseased and healthy plants respectively. Caldwell (2) has shown that

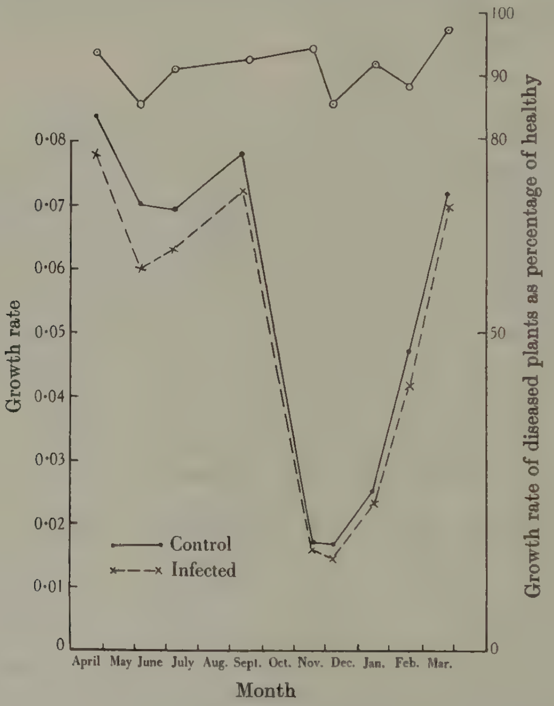

Fig. 3. Growth rate and time of year. Relative effect of virus on growth rate.

the water content of the leaves from a tomato plant at about the fourteen-leaf stage, infected with the virus of yellow tomato mosaic when a seedling, was 92·2 against 87·1 per cent. for the corresponding healthy leaves. An initial drop in water content of plants infected with these viruses does not seem to have been previously noted. Working with yellow tomato mosaic, Ainsworth found in the winter months at the time symptoms appeared, that infected seedling plants had a lower percentage water content than the controls (see Table II).

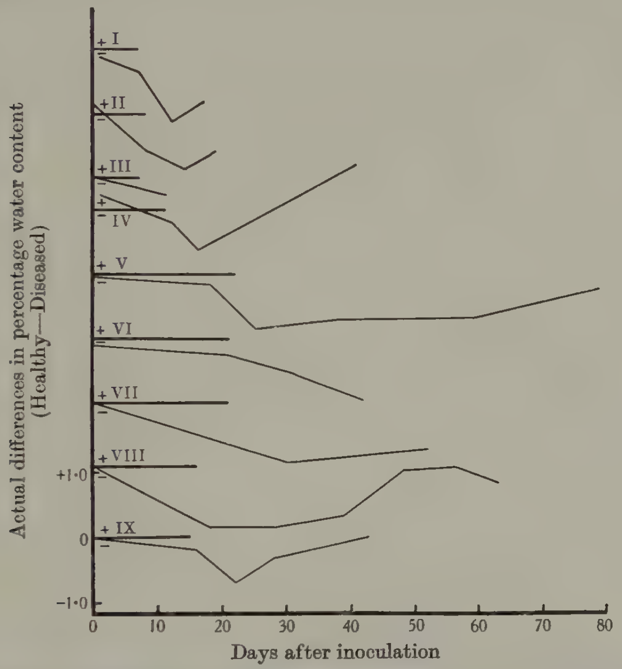

Fig. 4. Graphical representation of the differences between the percentage water contents of healthy and diseased plants shown in Table I. The horizontal lines indicate the length of the incubation periods. The ordinate scale for series IX only is given.

Table II

Exp.	Percentage water content (whole plant) Healthy	Infected (yellow mosaic)
1	94·55	93·35
2	94·35	92·50
3	92·60	91·25

Examination of the water content of the different parts of the plant suggested that the maximum fall was in the leaf and that the recovery starts in the root, continues in the stem and is evident last in the leaf. This is shown graphically in Fig. 5, which is based on the mean values from three comparable experiments (series I, II and III).

It is interesting to note that Melville (5) has demonstrated that in summer the percentage water content of the leaves of young greenhouse-grown tomato plants tends to be suboptimal for assimilation. The fall in water content initiated by the virus might further depress assimilation. On the other hand, during the winter months the water content of the leaves of tomato seedlings may be supra-optimal for assimilation, so that a reduction in water content at this time might be expected to favour assimilation. Nevertheless, the relative effect of the virus on growth rate appears to be the same during summer and winter.

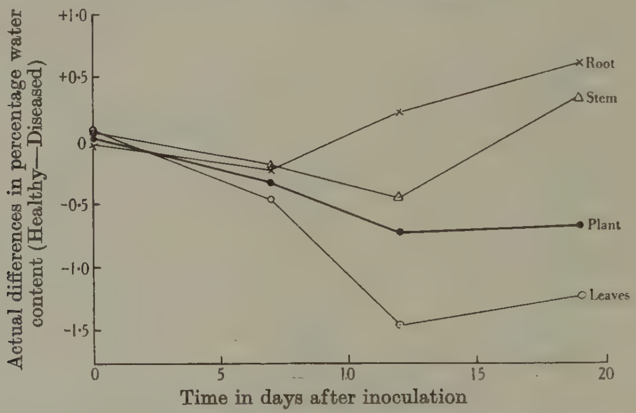

Fig. 5. Mean water content differences for stem, leaf, root and whole plant (series I, II, III).

The significance of the fall and rise of water content is obscure. The initial fall accompanies a slight "hardening" of the plant similar to that resulting from a check to growth such as a dry soil or low temperature. Withholding water from seedling tomato plants for 8 days resulted in a fall in the percentage water content which rose again when watering was resumed. Both fall and recovery of the water content were much more sudden than the similar disturbances due to tobacco mosaic virus. This experiment was carried out in February, the controls receiving water twice during the first 8 days.

Distribution of assimilate

If the stem and root are considered to play no part in photosynthesis, the ratio of the gain in dry weight of the stem and root to the gain in dry weight of the whole plant over any given period may be considered to give an indication of the distribution of assimilate. This ratio has been determined over the intervals between successive samplings, and no difference between healthy and diseased plants was found.

DISCUSSION

The results outlined above have shown that there is a high negative correlation between the growth rate of seedling tomato plants and the incubation period. They also suggest that the relative effect of the virus on the growth rate of the plant subsequent to infection is the same in both winter and summer, in spite of the great difference between the actual growth rates of healthy plants in the two seasons and the difference between the symptoms exhibited at these times.

One effect of this virus on both tobacco and tomato plants is to delay the hydrolysis of starch in the leaves. The presence of starch in the leaves of infected tomato plants after a period of darkness and its absence from the leaves of similar healthy plants may be demonstrated by treating decolourised leaves with iodine solution. Cordingly *et al.*(3) have concluded from chemical analyses that the hydrolysis of proteins and insoluble polysaccharides is delayed in the leaves of tobacco plants infected with *tobacco virus* 1.

Data obtained by W. H. Read at this Station, which he has kindly permitted us to publish, are of interest. From the analysis of samples of tomato leaves taken at 3-hourly intervals throughout a 24-hour period, he found that in plants infected with yellow mosaic virus, accumulation of starch during the morning was more rapid than in healthy plants. During the night the starch content of diseased and healthy leaves fell by 43·0 and 64·2 per cent., respectively. These results support the conclusions of Cordingly *et al.*(3).

This disturbance of the metabolism of the leaf might account for the reduction in growth, in the case of tobacco mosaic virus, which causes only a slight mottle in the summer and no mottle in winter. The accumulation of insoluble products in the leaf would tend to depress assimilation and their slower hydrolysis to reduce the absolute amount of material translocated. The distribution of the assimilate, as suggested above, does not seem to be affected, although there is a reduction in the total amount of dry matter produced. In the case of a virus such as that of yellow tomato mosaic, which in summer greatly reduces the amount of assimilatory tissue, delayed hydrolysis is certainly not the only factor reducing growth, although in winter when no yellow mottle is developed the conditions may be comparable to those obtaining with tomato mosaic.

SUMMARY

A negative correlation has been shown to exist between the growth rate of seedling tomato plants and the length of the incubation period.

Evidence has been presented, which suggests that the relative effects of *tobacco virus* 1 on the growth rate of the plant subsequent to the appearance of symptoms is the same in both summer and winter.

The percentage water content of all parts of infected plants was found to be lower than that of the controls, during the early stages of the disease. Later the water content tended to rise above that of healthy plants.

In conclusion we wish to express our thanks to Mr B. D. Bolas for his interest and valuable advice.

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THE ORIGIN OF AN EARTHY OR MUDDY TAINT IN FISH

I. THE NATURE AND ISOLATION OF THE TAINT

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Some forty years ago Rullmann (1, 2) isolated a type of *Actinomyces*, the cultures of which had a pungent odour, described by him and many others, including Beijerinck (3), as "earthy". Though this odour may not be identical with that of a well-aerated rich agricultural soil, as studied by Berthelot and André (4), it is sufficiently "earthy" to be regarded by those unfamiliar with the growth of actinomycetes as the odour of soil.

It was not until comparatively recently that this odour was connected with the presence of "earthy" tastes in potable waters. Adams (5, 6) drew attention to the occurrence of an "earthy" taste in water from the River Nile, and suggested that it was due to contamination of the water by actinomycetes, since he had been able to impart an "earthy" smell and taste to potable waters by adding cultures of actinomycetes.

Previously an "earthy" or "muddy" taste in water had been observed by several writers, including Rushton (7), and attributed to various diatoms found in the water. Subsequently Burger and Thomas (8) suggested that in a case studied by them the "earthy" taste was due to a slow complex microbiological decomposition of plants growing in the rivers from which the water under investigation was drawn. Burger and Thomas did not deny that odoriferous species of actinomycetes can impart an "earthy" taste to water, but preferred their conclusion that the taste resulted from the activity of a mixture of micro-organisms. They considered that the number of odoriferous actinomycetes found in the infected water was too small seriously to contaminate the water.

These various observations indicate that an "earthy" odour can be produced in water by micro-organisms living in the water, and that a pungent "earthy" taint can be imparted by certain odoriferous actinomycetes found in soil and in river water. The odoriferous substance produced by the actinomycetes is similar to that collected by Berthelot and André from agricultural soils, in that it is volatile in steam and slightly soluble in water; it is readily soluble in ether (Rullmann (1, 2) and Salzmann (9)). The material isolated by the writer from an odoriferous strain of *Actinomyces*, resembling in some of its characteristics the *A. flavovirens* of Waksmann, was partly soluble also in ethyl alcohol. The odoriferous substance was found to be an organic compound, possibly an organic amine; this in high concentrations had a distinct manurial odour, but in very high dilutions, preferably with slightly alkaline water, the odour became markedly "earthy".

The question of the production of an "earthy" odour by actinomycetes became of some practical importance recently, when it was reported that salmon caught in one of the richest salmon rivers of the kingdom had been found contaminated with an "earthy" taint which was particularly noticeable on boiling and which rendered the flesh of the fish quite uneatable.

When the complaint was brought to the writer's notice various suggestions had been put forward to explain the cause; one suggestion was that the fish in their progress up river had swallowed an excessive amount of mud introduced into the water by dredging operations some miles above the station where the affected fish were caught. The intestines of the contaminated fish examined, however, were free from mud and were, in fact, practically empty.

In a preliminary investigation of the problem, the staff of the Government Laboratory had observed that good drinking water in which sample pieces of tainted fish were cooked acquired an "earthy" taint, and that, on boiling, the odour was largely transferred from the fish and the water to the steam. These observations indicated that the "earthy" taint was due to some substance in the flesh of the fish, and that the substance was soluble in water and volatile in steam. It was observed also that water withdrawn from the river, even after filtration to remove suspended matter, had an "earthy" odour similar to that of the tainted fish.

At this stage of the investigation, after inspecting samples of the tainted water, the writer suggested that the "earthy" odour might be produced by certain actinomycetes. Later, the writer travelled by launch down the river from the tidal limit and examined samples of water, taken every few yards, for the presence of "earthy" odours. It was thought that, if growth of *Actinomyces* were the cause of contamination, the intensity of the odour would vary with the degree of *Actinomyces* development at the place of sampling.

The odour characteristic of *Actinomyces* growth was first noticed about ¼ mile below the upper limit of the tide. From this point over a distance of about 1000 yards down-stream, the water must have been badly contaminated since even the air above it was charged with the odour. The odour was particularly strong along one of the banks where mud, overgrown with reeds, had accumulated. Along the opposite bank the water was comparatively free from odour, but the taint was observed down the river, particularly in mid-stream, for more than a mile below the reed-covered mud bank. Above the tidal limit the water was free from odour, even in close proximity to the dredging operations, where the amount of suspended matter was higher than below the tidal limit.

Since the odour appeared to be confined to a comparatively restricted part of the tidal section of the river, it was decided to examine this part for odour-producing actinomycetes.

An exhaustive bacteriological analysis of the suspected section of the river was carried out with a view to determining the numbers and types of actinomycetes present. For this purpose samples of mud, water and decaying vegetation were taken at various distances below the tidal limit. Spore formation by actinomycetes in these samples was unlikely since the material had been permanently submerged, a condition which prevents spore formation by actinomycetes of group I of Ørskov (10), to which the odoriferous types belong. Each colony appearing on the culture media could, therefore, be assumed to represent an active focus of *Actinomyces* growth in the material and not merely one out of perhaps many million spores formed by a single focus of growth.

In Table I the results are given for the numbers of colonies of actinomycetes per gram of material which grew on the plates. Several types of *Actinomyces*, usually three or four, grew from each sample of material, but in all samples the odoriferous species prevailed.

Table I

	Left bank of river					Right bank of river			
No. of yards below tidal limit:	120	200	220	600	1,100	120	220	600	1,100
No. of *Actinomyces* per gm. of material:	280,000	220,000	609,000	330,000	400,000	8,400	870 (?)	500,000	362,000

The numbers were not high compared with the figures recorded in the literature for many soils (Waksmann (11)), but conditions in soil are hardly comparable with those in submerged river mud, since spore formation usually occurs extensively in aerated soils and one focus of *Actinomyces* growth may give rise to millions of spores each of which may be represented by a separate colony on the culture media, while under waterlogged conditions spore formation does not usually occur.

More instructive, therefore, than a comparison with aerated soils is one with the numbers obtainable from waterlogged materials of other rivers not contaminated by a perceptible "earthy" odour or taste. Material of this kind from the River Thames at Teddington gave 13,000 *Actinomyces* colonies per gram of submerged river mud, 52,000 colonies per gram of waterlogged vegetation and 244,000 colonies per gram of vegetation removed from the dry bank of the river. The number of actinomycetes in material from the worst stretch of the contaminated river were, therefore, from nineteen to fifty times higher than in the mud from the River Thames, and none of the species from the Thames mud was of an odoriferous type. The numbers in the samples from the contaminated river represent an abnormally high proportion of actinomycetes in the microflora.

The lowest numbers in Table I were obtained at two stations on the right bank, 120 and 200 yards below the tidal limit, where the odour of the water was slight. Along the left bank actinomycetes were numerous at all the stations examined, particularly at 220 yards (near the point of discharge of sewage effluent into the river) where the earthy odour of the water was strong. The actinomycetes at this place appeared actively to destroy the submerged reeds. In one instance the bases of the submerged reeds yielded as many as 520,000 actinomycetes per gram while the surface mud at the same place gave only 280,000 per gram. In another place the bases of the reeds yielded 760,000 actinomycetes per gram and the mud 4 in. below the surface less than 1000. Surface mud from another part of the bank of reeds yielded 400,000 colonies per gram, while surface mud taken 5 yards beyond the bank towards mid-stream harboured no more than 86,000 per gram. It was concluded, therefore, that the whole of this reed bank was an important seat of *Actinomyces* activity. Activity of actinomycetes was not, however, restricted to this bank; a thorough examination of the banks of the whole of the tidal part of the river as well as of the surrounding fields revealed very high numbers of odoriferous actinomycetes, which may have contributed some part of the odoriferous substance dissolved in the river water.

The next point to investigate was whether the "earthy" taint in the river water, caused by actinomycetes, could be imparted to fish living in the water and, if so, how such contamination of the fish occurred. Salmon, which enter the river from the sea, may remain for only a brief period in the tidal reaches. If, therefore, they become tainted with the "earthy" odour, the transference of the odour to the fish must be fairly rapid. For this reason it seemed likely that the passage of the odour into the fish took place through the gills and that it was carried with the blood stream to the various tissues, notably the muscles. An account of the investigation carried out to ascertain the effect of *Actinomyces* odour on fish is given in the second paper of this series in which it is shown that an "earthy" taint in the flesh can be produced experimentally in the laboratory. For the purpose of these experiments, the suspected strain of *Actinomyces* was grown in broth of the composition ordinarily used in bacteriological work. After 5 days' growth the broth had acquired a strong *Actinomyces* or "earthy" odour. It was found that by distillation of the broth at ordinary pressure, the odoriferous substance could be transferred in concentrated form to the distillate. The distillates used in the experiments with fish were prepared from broth in which the *Actinomyces* had grown for 20 days; they gave a strong positive reaction with Nessler's reagent and contained ammonia in addition to the odoriferous substance. This substance could be separated from the ammonia by extraction of the distillate with ether in which it was soluble. After removal of the ether, the concentrated substance consisted of a brown amorphous material with a strong manurial smell. When diluted two parts in ten million parts of water of a slightly alkaline reaction (pH 7·5–8·0) the substance had a typical *Actinomyces* or "earthy" odour. Dilutions of the ether-extracted substance equivalent to 1 part and 5 parts per million parts of water were used for the experiments with fish; they represented concentrations approximately equal to those in the contaminated river water.

SUMMARY

Certain types of *Actinomyces* produce a pungent odour which has frequently been described as "earthy". This odour is caused by an organic compound which is slightly soluble in water, volatile in steam, soluble in ether, and partly soluble in alcohol. In concentrated form the substance forms a brown amorphous material with a penetrating manurial odour.

When dissolved in water in concentrations as low as two parts in ten million it imparts to the water a pungent *Actinomyces* or "earthy" taint which is particularly noticeable when the reaction of the water is maintained on the alkaline side of the neutral point.

The existence of an excessive contamination of an important salmon river with odour-producing actinomycetes was established. This con-

tamination and the resultant pollution of the water with a pungent "earthy" taint was restricted to the tidal part of the river, where decaying organic matter was abundant.

Acknowledgment is due to the Inspector of Salmon Fisheries for Scotland for his interest in the work. Thanks are also due to the Director of Chemical Research in the Department of Scientific and Industrial Research for facilities afforded in publication of the results of these experiments with which the Water Pollution Research Board of the Department has also been concerned.

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THE ORIGIN OF AN EARTHY OR MUDDY TAINT IN FISH

II. THE EFFECT ON FISH OF THE TAINT PRODUCED BY AN ODORIFEROUS SPECIES OF *ACTINOMYCES*

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and F. T. K. PENTELOW
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In the preceding paper (1) one of the writers (A. C. T.) reported the isolation from an important salmon river of a species of *Actinomyces* which produced a pungent "earthy" odour. The odour was due to an organic compound produced by the organism during growth. The compound was volatile in steam, soluble in ether, partly soluble in alcohol and slightly soluble in water; it could be isolated by distilling cultures of the organism and extracting the distillate with ether; thus obtained it was a brown amorphous mass of distinct manurial odour. When diluted one part in five million parts of water it imparted to the water a distinct "earthy" taint apparently identical with that in the tainted river water. The odoriferous substances from the cultures and in the river water were both volatile in steam and nauseatingly "earthy".

In the earlier paper it was suggested that fish travelling through water contaminated by the substance with an "earthy" odour, such as that produced by odoriferous actinomycetes, would absorb the substance as the water passed through their gills and would distribute it with the blood stream throughout their bodies where it might accumulate in the muscular tissues. It was thought that this mode of contamination would be more likely to lead to a rapid tainting of the flesh of the fish than other methods suggested, namely that fish in their passage through the contaminated water would swallow large quantities of river mud, or that a deposit of river mud on the skin of the fish would lead to tainting of the flesh. The suspended mud in the river water, however, did not possess the peculiar "earthy" odour of the affected fish.

For the purpose of ascertaining whether the flesh of fish in contact for short periods with water tainted by the odoriferous substance produced by actinomycetes would also become tainted, several series of experiments, which are described in the following paragraphs, were carried out. As salmon could not be obtained for the trials brown trout and rainbow trout were used.

In the first series a stock of brown trout was procured from a commercial hatchery. These fish as received had no "earthy" taint and remained free from any suspicion of the taint when stored in a stock pond at the Fisheries Research Station, Alresford.

A solution of the distillate, prepared as described in the preceding paper (1) from cultures of the odoriferous *Actinomyces*, was diluted with water from the River Itchen in the proportions of 1 of distillate in 5 of the mixture, 1 in 10, 1 in 20, 1 in 100 and 1 in 200, giving concentrations of odour-producing substance of the order of 40, 20, 10, 2 and 1 parts per million respectively. In the two highest concentrations the fish showed symptoms of poisoning, but this was mainly if not entirely attributable to high concentrations of ammonia in the distillate; other experiments showed ammonia to be highly toxic to fish. In all instances the fish were removed after 1 hour, killed, washed thoroughly and cooked by boiling rapidly for a few minutes in water containing a small quantity of common salt.

In every instance the flesh of the fish had acquired a marked "earthy" odour and flavour. In those fish subjected to dilutions with water of 1 of distillate in 5, 1 in 10 and 1 in 20, that taint was nauseatingly strong. The more dilute solutions produced a taint of the same nature, but it was not so strong though the flesh was definitely unpalatable. The taint could be detected not only in the cooked but in the uncooked flesh and in the water in which the fish were cooked. Fish from the same stock pond, subjected to the same treatment except that no distillate was added to the water, did not acquire any "earthy" taint.

An experiment was then carried out, using the brown amorphous substance separated from the distillate by extraction with ether. This substance, when dissolved in River Itchen water in the proportion of 5 parts per million, imparted to trout in 1 hour the same degree of taint as that produced by solutions of the distillate of approximately the same concentration (1 in 40).

In the course of later experiments it was found that rainbow trout recently obtained from a hatchery had, when cooked, a distinct "earthy" taste of the same nature as that in fish which had been subjected to exposure to the distillate from *Actinomyces*. A visit was therefore paid to the pond in which these fish had been stored and samples of mud and water were taken. These samples had a distinct "earthy" odour, and bacteriological examination showed that they contained odoriferous *Actinomyces* amounting in numbers to approximately 19 per cent. of the total microflora.

A stock of rainbow trout from this hatchery was stored in a pond at the Fisheries Research Station, Alresford, for about a week. At the end of this period the taint in the killed and cooked fish was imperceptible. A sample of the mud from the pond containing the *Actinomyces* taint was mixed with River Itchen water and a rainbow trout was placed in the mixture for 1 hour. At the end of that time the fish was killed, cleaned and washed. An "earthy" odour was perceptible in the flesh of the fish, and after cooking the fish had a distinct "earthy" taint, which could also be detected in the water used for cooking. This taint was similar to that produced by the brown amorphous substance isolated from distillates of cultures of the *Actinomyces* from the infected salmon river previously investigated.

In another experiment trout were exposed to a heavy suspension of mud free from the odour associated with actinomycetes. In this experiment, although the fish were left in contact with the suspension for 4 hours, no "earthy" taint could be detected, either by taste or smell, in the uncooked or cooked flesh or in the water in which the fish were cooked.

From these experiments it appears reasonable to conclude that the "earthy" taint sometimes found in the flesh of fresh-water fish is due not to mud but to a substance derived from an odoriferous species of *Actinomyces*, which infects the water in which the fish live.

From the rapidity with which the "earthy" taint permeated from the water into the flesh of the fish it seemed probable that it entered through the gills and was carried into the muscles of the fish by the blood stream. A series of experiments was carried out to test this hypothesis.

It was found that dead fish when placed for 1 or 2 hours in a solution of 2 parts and 20 parts per million of the ether-extracted odour-producing substance acquired no taint, although live fish were strongly tainted after 1 hour. In the course of some confirmatory experiments it was found that one trout, although it had been dead for 1 hour, became strongly tainted when immersed in a solution of 20 parts of the active substance per million, but in this instance one of the larger blood vessels in the gills had been ruptured and it is possible that the taint may have entered through the broken blood vessel. Further experiments confirmed the original observation that uninjured dead trout do not acquire the taint.

By the use of a rubber balloon it was found possible to confine a trout so that its head and gills were exposed to one solution and its body to another. The narrow end of the balloon was distended and bound tightly to the open end of a cylindrical aluminium case. The fish was inserted through the stretched neck of the balloon into the cylinder. When the neck of the balloon was released it fitted tightly round the fish in the region of the pectoral fins, and liquid in the cylinder was completely isolated from the outside. By this method it was possible to permit fish to breathe clean water while their bodies were immersed in a solution of the odoriferous substance, or to breathe the solution of active substance whilst their bodies were in clean water. In these experiments it was found that fish breathing tainted water invariably acquired a strong "earthy" taste, while those breathing clean water but with their bodies exposed to the solution of *Actinomyces* extract never acquired a taint.

These experiments strongly suggest that the taint enters the fish principally through the gills and is carried to the muscles by the blood stream. There is no evidence that tainting can occur through the skin though it is possible that the taint might be acquired from water taken into the stomach. This could not be determined.

THE REMOVAL OF THE TAINT

In cookery books it is generally recommended that fresh-water fish taken from muddy rivers or ponds should be kept in running water for a few days before they are required for the table "in order to remove the muddy taste". It was found experimentally that eight trout which had been exposed for 1 hour to a solution containing 10 parts of the odoriferous substance per million parts of water and which had acquired a strong "earthy" taint lost this taint gradually when kept in clean running water for some time. Seven of these tainted fish were placed in a tank of about 140 litres capacity supplied with clean River Itchen water at the rate of approximately 500 c.c. per min. The fish were removed one by one at intervals of 4 hours, 10 hours, 22 hours, 27 hours, 2 days, 3 days, and 5 days. Up to 27 hours no diminution of the taint was apparent, but it then became less marked and in the fish killed after 2 and 3 days it was definitely weaker though still obvious. In the fish killed after 5 days no taint could be detected. In another experiment three rainbow trout were exposed to the mud containing *Actinomyces* growth for 2 hours. One fish was then killed and found to be distinctly tainted. The other two fish were transferred to a small tank of a capacity of 33 litres supplied with River Itchen water at a rate which varied from 500 to 900 c.c. per min., averaging about 750 c.c. per min. One fish was found dead after 2 days and cooked. The taint was still distinct. The other fish was killed after 5 days and was found to be quite free from any muddy taste.

These preliminary experiments indicate that the rapidity with which the taint is acquired is in marked contrast to the rate of its removal by clean water.

The Inspector of Salmon Fisheries for Scotland kindly arranged for some of the prepared extract from odoriferous *Actinomyces* to be employed in experiments with sea trout. The results of these experiments were similar to those obtained with brown trout in the experiments described in this paper.

SUMMARY

1. A muddy or "earthy" taste can be imparted to the flesh of trout by material produced by an odoriferous *Actinomyces*.
2. The taste resembles that imparted by a natural mud rich in actinomycetes.
3. The taint appears to be acquired through the gills and to be carried in the blood stream.
4. The taint can be slowly removed by clean flowing water.

The authors wish to express their thanks to the Director of Fishery Investigations of the Ministry of Agriculture and Fisheries for permission to publish these results, and to the Inspector of Salmon Fisheries for Scotland for his interest in the work. Acknowledgment is also due to the Director of Chemical Research in the Department of Scientific and Industrial Research for facilities afforded in publication of the results of these experiments with which the Water Pollution Research Board of the Department has also been concerned.

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THE SORGHUM MIDGE IN THE ANGLO-EGYPTIAN SUDAN

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(With Plate IV)

Introduction and history

Sterility or partial sterility of the heads of dura, *Sorghum caudatum* Stapf., has been seen in varying intensity for several years in the Blue and White Nile Provinces of the Anglo-Egyptian Sudan (Plate IV A, B). Severe losses of a similar nature are caused in the United States of America by *Contarinia sorghicola* (Coq.) in all the principal sorghum-producing areas, particularly in the Gulf States (1).

This condition of sterility is well known to the natives by the name "Masa", but native opinion differed as to the cause. Heavy rainfall while the crop was in flower had been blamed, and also lack of rain at a critical period of plant growth. "Masa" is generally worse in years of heavy late rains, but as a single head of dura takes many days to complete its flowering, no meteorological factor could account for the complete sterility of heads over large areas, as conditions vary widely at that period.

Punter (2), while working on sorghums, was the first to point out that midge larvae were the cause of the damage in the Sudan, and this observation has been fully confirmed. There is no doubt that "Masa" damage has often in the past erroneously been attributed to the Pentatomid bug, *Agonoscelis versicolor* F.

Identity

Specimens of the adult midges were first sent to the Imperial Institute of Entomology for identification in 1932 by Mr H. B. Johnston. Dr H. F. Barnes of Rothamsted Experimental Station, who examined the specimens (Barnes Collection, Cecid. 2095–7), stated that they were a species of *Contarinia*, and drew attention to the fact that certain species of this genus, notably *C. sorghicola* (Coq.), were known as pests on various species of *Andropogon*, preventing seed production in the U.S.A., India and Italy. Later, in November 1934, the present writer submitted specimens of larvae, pupae and adults to Dr Barnes for determination. He tells me now that, thanks to the kindness of Dr E. P. Felt, Director of the Bartlett Tree Research Laboratories, Stamford, Conn. and of Mr E. V. Walter, in charge of the U.S. Federal San Antonio Station, he has been able to compare my material (Cecid. 2385–93 and 2505–8) with specimens (Cecid. 2509–23) of *Contarinia sorghicola* (Coq.) from San Antonio, Texas. He can find no constant differences between the specimens from the Sudan and those from Texas, therefore the Sudanese midge can be considered to be *C. sorghicola* (Coq.). Dr Barnes has pointed out to me, however, that, as certain species of the genus *Contarinia* are morphologically so similar, the final test of conspecificity must be interbreeding experiments.

DESCRIPTION

The fly measures about 2 mm. in length, the wings when closed extending just beyond the tip of the abdomen. Its slender body is bright red with dark plates across the terga. Antennae are brown and very long. Head, thorax and legs dark brown. Wings transparent, but slightly dusky in colour. The female is slightly larger than the male with a long ovipositor which enables her to deposit her eggs within the flowering spikelets.

The egg is elongate in form, orange yellow in colour and measures 0·4 mm. in length and 0·1 mm. in width.

The larvae are typical midge larvae, at first creamy white, but soon suffused with orange red, with small darkened mouthparts. When full grown, the larvae reach a length of 1½ mm. and become redder.

The pupae are about 2 mm. in length, bright red in colour with dark head, thorax, wing cases and legs.

The resting larvae are enclosed in a puparium surrounded by a few strands of silk. The colour of the puparium is a light tan.

DISTRIBUTION

The midge, as far as at present known, occurs in a belt running east and west through southern Kassala, Blue and White Nile Provinces. The southern and northern limits are at present unknown.

LIFE HISTORY

All stages can be found in the field from the end of September until the end of December. Adults from the resting generation emerge during the latter part of the rains.

The eggs are laid in the spikelets on the inner wall of the glumes or between the pales. Oviposition takes place at a very early stage while the anthers are still enclosed within the spikelets, or as soon as they extrude. The eggs are laid singly, although as many as six have been found in one spikelet, probably laid by several females.

The larvae obtain their nutriment from the young grain whose further growth is completely arrested. One larva is sufficient to cause the abortion of the grain in a spikelet, but as many as three have been known to mature in one spikelet.

Considerable red staining occurs on the shrivelled grain and pales of the spikelet during midge attack.

The larvae, when full fed, pupate in the spikelet, and adults emerge a few days later. There is generally a high preponderance of females. The females are most active in the morning and can be observed busy ovipositing on the spikelets.

At the end of December, or early in January, the larvae within the spikelet form cocoons and remain within as resting larvae. In this way they are able to pass over until the following season.

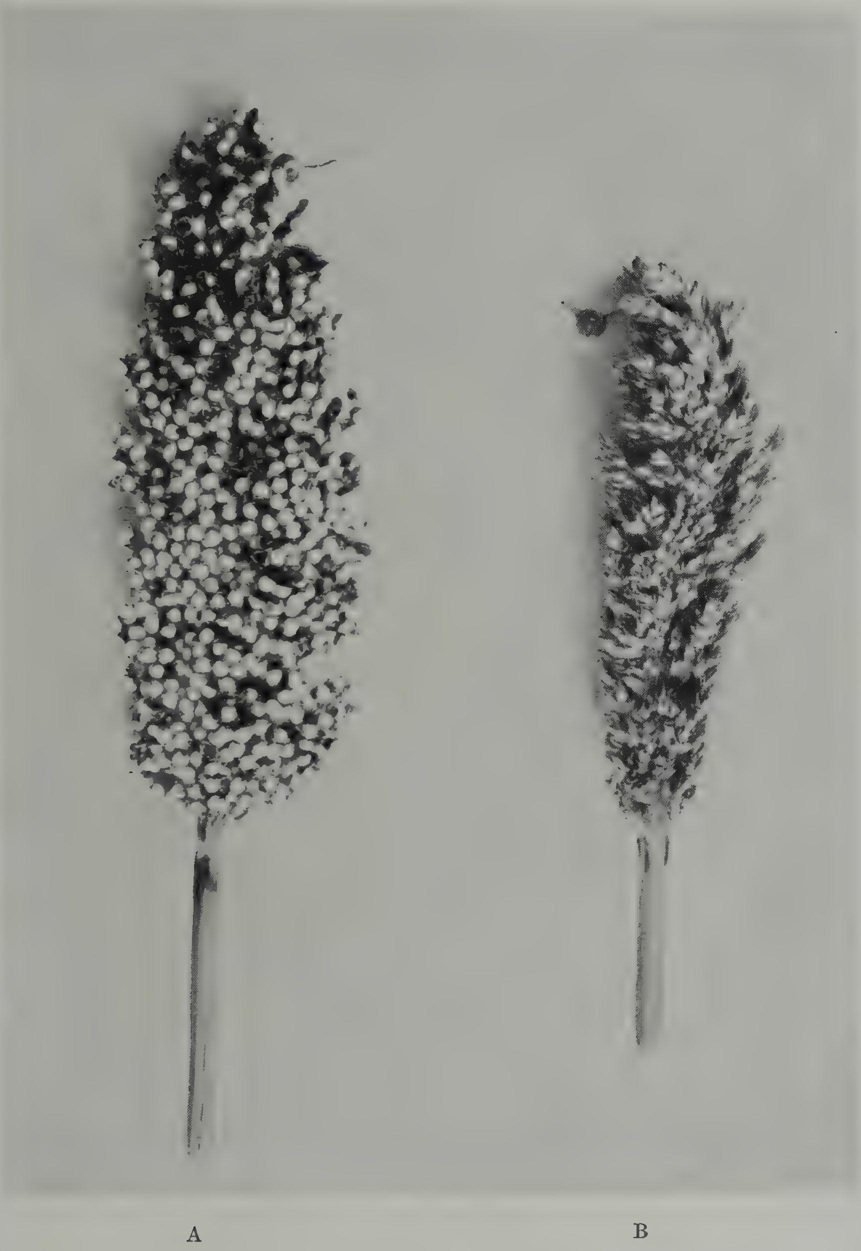
Damage and control

No damage by this midge has been noticed on dura flowering before the middle of September, but it can be very severe, especially to late-sown dura or heavy tillering varieties after that date. No variety of dura escaped infestation by the midge, although there seem to be degrees of resistance. The grass "Addar" (*Sorghum halepense* Pers.) is also infested in this area.

In the Blue Nile Province, Feterita dura is the main variety and is sown either on rain land as soon as sufficient rain has fallen to germinate the seed, or on irrigated land. In both cases sowing normally takes place about the end of July, or early in August. The plants flower 40–50 days after sowing.

The damage varies from year to year but reductions of $1\frac{1}{2}$ ardebs (1 ardeb = 336 lb.) of grain per feddan or a 25 per cent. loss is not uncommon.

The main heads of dura in most years practically escape injury, but in some years especially when much resowing takes place a large proportion of the heads may be rendered more or less sterile. Damage is nearly always severe in late tillering shoots. Those flowering during October may suffer as much as 50 per cent. sterile heads, while those flowering in November may be a complete loss.



A B

COWLAND.—The Sorghum Midge in the Anglo-Egyptian Sudan (pp. 110–113).

Until more detailed information is available regarding the biology of this insect, no definite control measures can be recommended. There are strong indications that by early and close sowing of a non-tillering variety of dura, a good crop can be obtained before the midge has become numerous. As, however, the possibility of early sowing is dependent on a sufficiency of rain at an early date, this measure cannot be relied on.

Summary

The sorghum midge, *Contarinia sorghicola* (Coq.), is reported for the first time from the Anglo-Egyptian Sudan.

A short account is given of the distribution, life history, damage and control of this midge as it occurs in the Anglo-Egyptian Sudan.

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EXPLANATION OF PLATE IV

Heads of Sudan Feterita dura; A, healthy; B, sterile, attacked by larvae of the sorghum midge, *Contarinia sorghicola* (Coq.).

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SOME NOTES ON THE MORPHOLOGY OF THE IMMATURE STAGES OF SOME BRITISH CHAFER BEETLES

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(With 7 Text-figures)

Introduction

Genera of the subfamily Melolonthinae (Coleopt.) which occur in the British Isles number in all only five, namely *Hoplia*, *Homaloplia*, *Serica*, *Amphimallus* and *Melolontha*; of these, the three latter are of considerable economic importance. In spite of this fact, little work seems to have been done on the morphology of the immature stages of these pests.

The following descriptions are primarily intended therefore as a means of identification of the species, but since the three commonest of these, namely, *Melolontha melolontha* L., *Amphimallus solstitialis* L. and *Serica brunnea* L., have received little attention since Schiodte (11) and Perris (8) figured them over fifty years ago, a detailed study has been carried out in the light of more modern conceptions of morphology. A description of the internal structures has not been undertaken, since it seemed to be of less economic importance. Edwards (3) and Dufour (2) have both, however, made very close studies of the internal morphology of larvae of the Scarabaeoid type, and it seems probable that the larvae of the Melolonthinae are very similar to those they have studied.

The descriptive text of the paper is divided into sections dealing with each of the different stages of the insects, and the subject is treated from a comparative point of view. In the summary an identification table is drawn up, including most of the Scarabaeoid larvae which cause damage to crops in this country. This is intended only for rough practical use, since for complete certainty in identification reference must be made to the fuller descriptions.

Details of the adult stages are not included, since these can be found adequately described and figured in such text-books as Fowler (5).

A. EGG STAGE

The eggs of all three species are ovate in shape, being rather elongate when first laid, but becoming rather broader just previous to hatching. In colour they are white to creamish, those of *Serica* appearing a little whiter than those of the other two species. The surface of the egg in *Amphimallus* and *Serica* is rather dull and matt, although it appears quite smooth under the microscope. With *Melolontha*, on the other hand, the surface is distinctly granulose, being covered with small pits very reminiscent of the stridulating area on the larval mandibles of this species.

The chorion of the eggs is transparent and the embryo larva can be distinguished through it. The surface is usually covered with a sticky substance secreted by the colleterial glands. The sizes of the eggs do not vary a great deal from the mean for each species, although these differ widely. Table I shows the average dimensions of the eggs soon after they are laid.

Table I

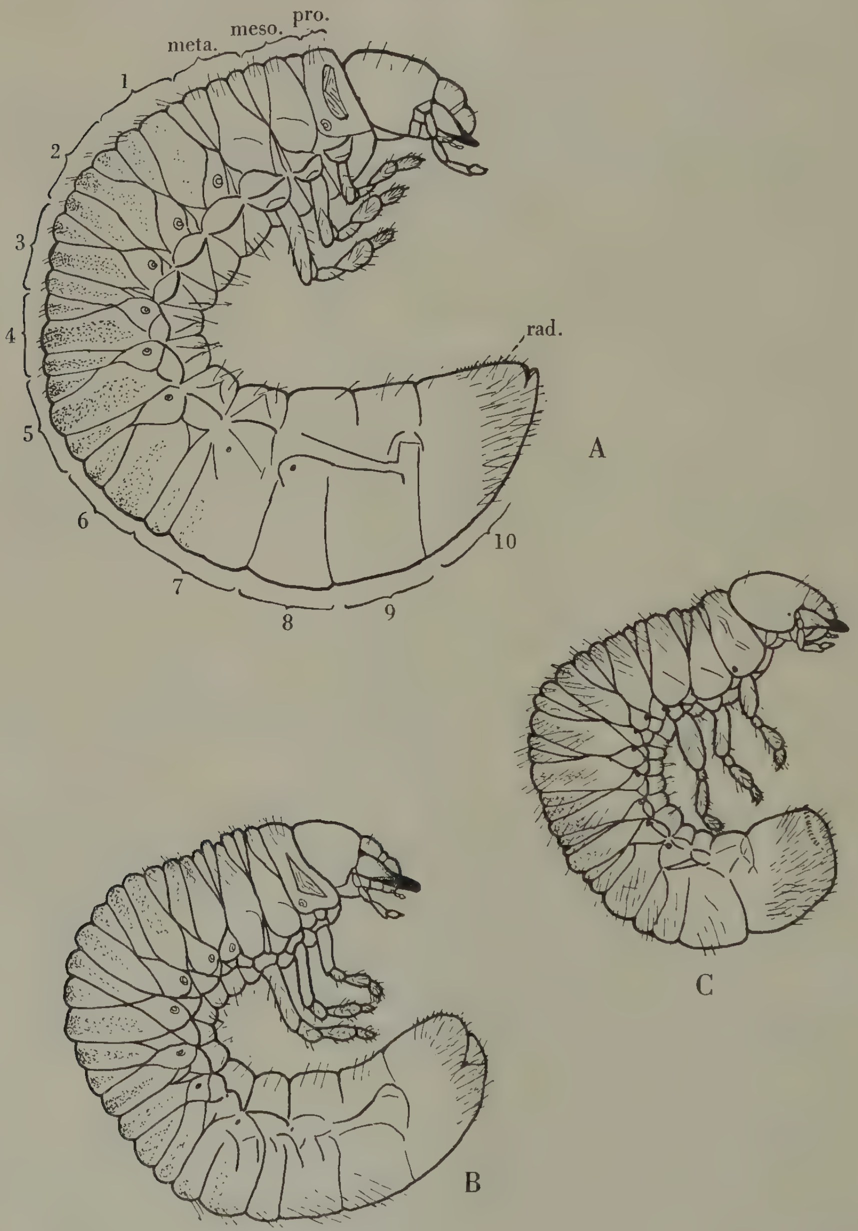
Average dimensions of eggs, in mm.

	Width	Length
M. melolontha	2·39	2·92
A. solstitialis	1·37	2·16
S. brunnea	1·27	1·53

B. LARVAL STAGE

The larvae of the Pleurosticti can be separated from those of the Laparosticti, according to Erichson (4), by the fact that the lobes of the maxillae are connate instead of being separate. The former group is again divided by this writer into larvae of the Cetonine-Dynastine type which have mandibles obtusely dentate at the apex, and furnished with transverse striae on the posterior surface, and those of the Melolonthine type which have mandibles with one smooth tooth at the apex and an unfurrowed posterior surface.

In general appearance the larvae of all the Scarabaeoid species are very much alike, the thorax and abdomen, which are soft and fleshy, being ochreous white in colour, and the head a darker brown. When the larvae are well fed the food can often be seen through the semi-transparent viscera as dark brown or black at the posterior end of the abdomen. The body, which is elongate and round in section, is usually curved in a semicircle in a ventral direction, so that the radula is almost touching


pro.
meso.
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1
2
3
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5
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7
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9
10
rad.
A
C
B

Fig. 1. Fourth instar larvae, from the right side. A, *Melolontha melolontha*, L. × 2½; B, *Amphimallus solstitialis*, L. × 3¼; C, *Serica brunnea*, L. × 6½. *meso.* mesothorax; *meta.* metathorax; *pro.* prothorax; *rad.* radula; 1–10, segments of abdomen.

the mouth parts (Fig. 1 A, B, C). If the larvae of any of these species are placed on a smooth flat surface, they will in most cases be able to crawl about on their legs with the abdomen stretched out behind; *Melolontha*, however, is unable to do this, apparently because it cannot straighten the body enough. *Cetonia* also seldom crawls on its legs, but moves on its back by means of the caudally pointing hairs thereon, using the legs only for digging.

The thorax may in most cases only be differentiated from the abdomen by the presence of three pairs of thoracic legs. The abdomen also varies slightly in shape in the different species, in some being club-shaped and slightly pointed at the caudal end, while in others it is blunt and rounded.

The different portions of the mature larvae of the three commonest species of the British Melolonthinae, as defined above, will be described in detail in the following pages. The present writer has been unable to note any marked morphological differences between the separate instars of a species, other than the gradual increase in size. The forms of the spiracles and labrum-epipharynx, which might be expected to vary, are in every case identical right through the larval stages; also there is no sign of an egg burster such as Ritterschaus (9) describes in the first instar of *Phyllopertha*. There is, nevertheless, an increase in the number of spines in the radula in each succeeding instar; a fact which helps to confuse the early stages of *Melolontha* with the more mature ones of *Amphimallus* and *Phyllopertha*. The post-embryonic changes in the Scarabaeoidea have been further studied by Grandi (6).

The larva of *Hoplia*, of which there are few very detailed descriptions available, may be recognised by the following characters: fourth joint of the antenna half the length of the third; anal orifice transverse and slightly angulate; posterior half of the last abdominal segment having on the ventral side two parallel rows of spines covering more than three-quarters of this part; on either side of these rows are small areas of scattered bristles; this segment is irregularly and lightly fluted on the dorsal side.

(1) *Head*

The head (Fig. 2) of the larva is the most heavily chitinised portion of the body and the darkest in colour. In all three species the colour is light ferruginous, shading into black at the distal corners of the clypeus and the tips of the mandibles. The head is borne at right angles to the long axis of the body, with the mouthparts pointing ventrally, but in

Serica the ventral side of the head is directed a little more anteriorly than in the other two species.

In gross anatomy the heads of all three species are very similar, and only vary slightly in shape and the relative proportions of the parts.

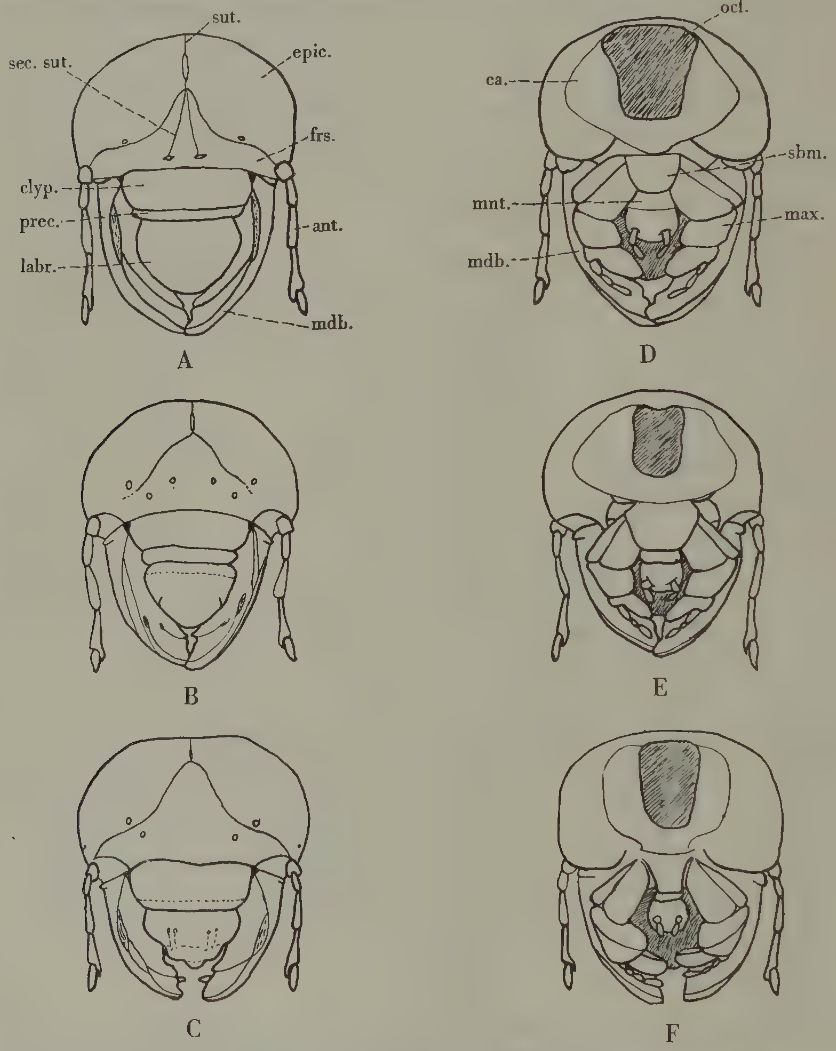


Fig. 2. Anterior and caudal aspects of larval head. Anterior: A, *Melolontha*; B, *Amphimallus*; C, *Serica*. Caudal: D, *Melolontha*; E, *Amphimallus*; F, *Serica*. *ant.* antenna; *ca.* cervical area; *clyp.* clypeus; *epic.* epicranium; *frs.* frons; *labr.* labrum; *max.* maxillae; *mdb.* mandible; *mnt.* mentum; *ocf.* occipital foramen; *prec.* preclypeus; *sbm.* submentum; *sec.sut.* secondary suture; *sut.* epicranial suture.

Serica has perhaps the broadest head capsule, being slightly flattened dorsally, while *Amphimallus* has the narrowest in proportion to its length. The epicranium (*epic.*) is divided dorsally by a suture (*sut.*) running longitudinally and branching Y-shaped, passing in a ventral direction round the borders of the frons (*frs.*). In *Melolontha* there is a secondary suture (*sec.sut.*) or depression which Hayes (7) considers to be a vestigial suture disappearing in the course of specialisation. This suture is difficult to locate in *Amphimallus* and wanting in *Serica.*

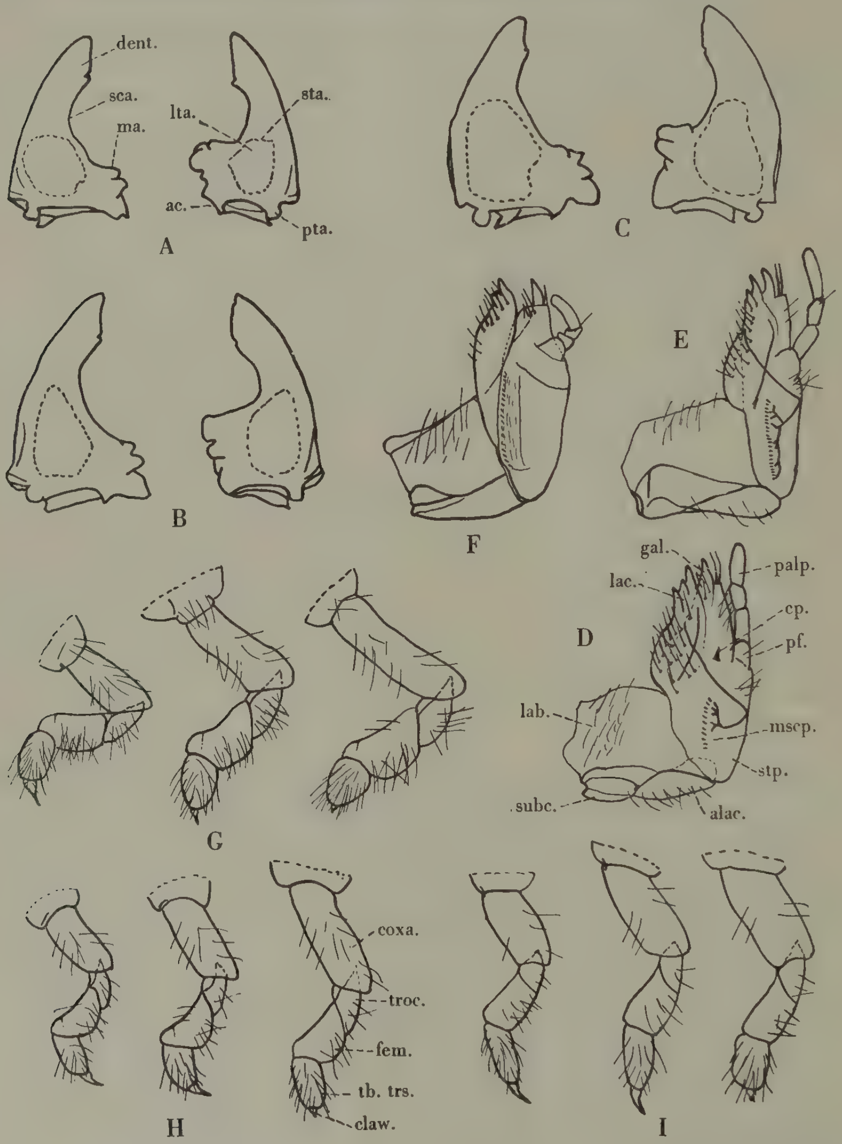
Situated on the anterior side of the head capsule are a number of small depressions, all three species having a pair on the epicranium just dorsal to the branches of the epicranial suture. In *Melolontha* there is another pair at the ventral end of the secondary sutures, and in *Amphimallus* two pairs on the frons, one pair being slightly dorsal to the other. *Serica* is similar to the latter except that the dorsal pair on the frons is wanting. From a caudal view, the head presents a large occipital foramen (*ocf.*) which varies in shape in each of the three species. Round this is a slight ridge enclosing an area (*ca.*) on which are attached the muscles and membrane of the cervix. Ocelli seem to be entirely wanting, although in *Serica* there is a small patch of pigmented cells just dorsal to the base of the antennae. These do not seem to have been noted by other writers, and it is unknown whether they are sensitive to light or not.

(2) *Antennae*

The antennae (Fig. 4 A, B, C) are situated on the head in a position slightly caudal to the base of the mandibles. Most writers now consider the antennae to be made up of five joints, instead of four as was originally thought. The first joint, which is short and boss-like, is more or less fused with the head. The next three joints are longer and their relative lengths vary in the three species. In *Melolontha* all three are about the same length; in *Amphimallus* the third joint is the longest and the second is shorter than the fourth; in *Serica* the third is also the longest, but the second and fourth are equal in length. The fifth joint is short and pointed, with a group of sensillae at the tip and three sensory patches on the sides. In all three species the fourth joint bears on its distal side a process which projects forward about one-third the length of the fifth joint.

(3) *Labrum-epipharynx*

Although of great taxonomic importance, the epipharynx of the European Scarabaeidae has not received a great deal of attention. Schiodte (11) figured that of *Geotrupes*, and Ritterschaus (10) has described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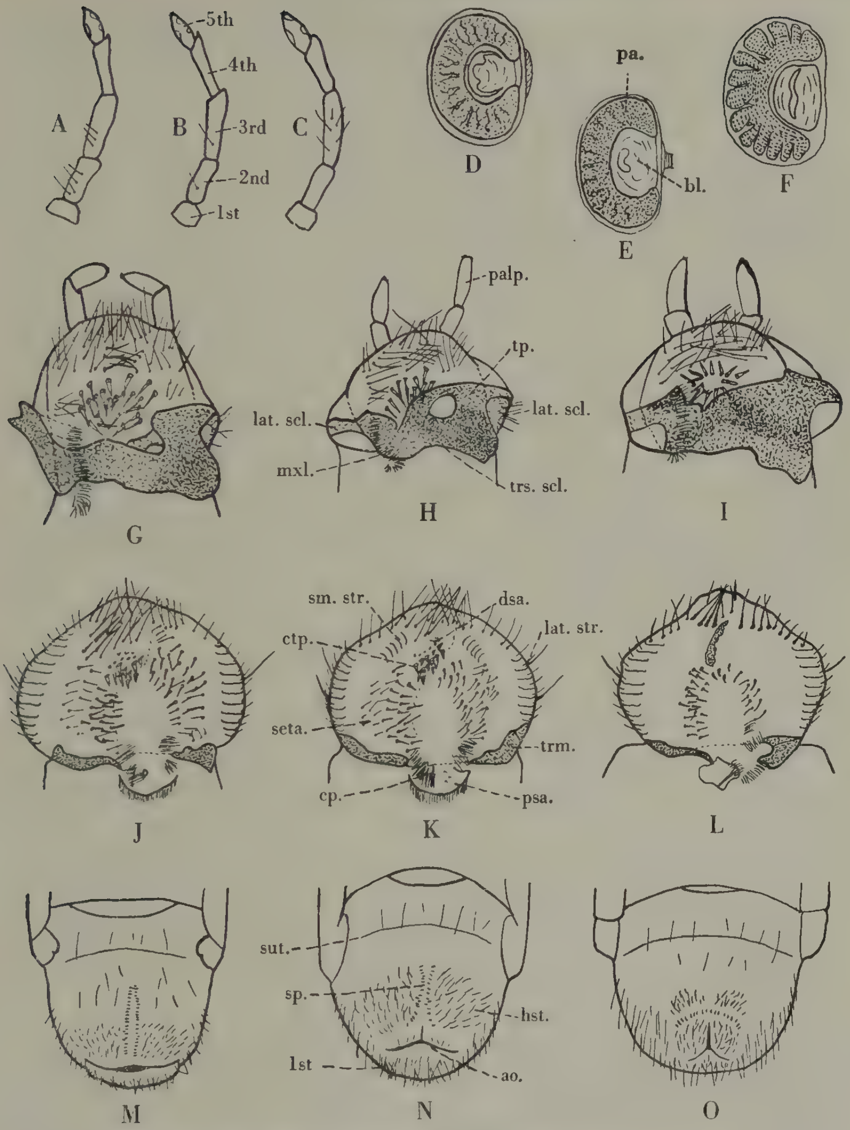
mscp.
stp.
subc.
alac.
G
coxa.
troc.
fem.
tb. trs.
claw.
H
I

Fig. 3. Appendages of the head and thorax. Caudal aspect of mandibles: A, *Melolontha*; B, *Amphimallus*; C, *Serica*. Cephalic aspect of right maxilla: D, *Melolontha*; E, *Amphimallus*; F, *Serica*. Left thoracic legs: G, *Melolontha*; H, *Amphimallus*; I, *Serica*. *ac.* acia; *alac.* alacardo; *cp.* chitinous pegs; *dent.* dentes; *fem.* femur; *gal.* galea; *lab.* labacoria; *lac.* lacinia; *lta.* light area; *ma.* molar area; *mscp.* maxillary scraper; *pf.* palpifer; *pta.* postartis; *sca.* scissorial area; *sta.* stridulating area; *stp.* stipes; *subc.* subcardo; *tb.trs.* tibio-tarsus; *troc.* trochanter.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lat. scl.
mxl.
trs. scl.
G
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dsa.
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Fig. 4. Appendages of the head and abdomen. Right antenna: A, *Melolontha*; B, *Amphimallus*; C, *Serica*. Left prothoracic spiracle: D, *Melolontha*; E, *Amphimallus*; F, *Serica*. Hypopharynx: G, *Melolontha*; H, *Amphimallus*; I, *Serica*. Epipharynx: J, *Melolontha*; K, *Amphimallus*; L, *Serica*. Ventral aspect of abdominal segments 9–10: M, *Melolontha*; N, *Amphimallus*; O, *Serica*. *ao.* anal orifice; *bl.* bulla; *cp.* chitinous plate; *ctp.* chitinous pegs; *dsa.* distal sensory area; *hst.* hooked setae; *lat.scl.* lateral sclerites; *lat.str.* lateral striae; *lst.* long setae; *mxl.* maxillula; *pa.* perforated area; *psa.* proximal sensory area; *sm.str.* submarginal striae; *sp.* spines; *sut.* suture between segments 9 and 10; *tp.* toothed projection; *trm.* torma; *trs.scl.* transverse sclerite.

those of *Anomala aenea* De G. and *Phyllopertha horticola* L. in great detail; also Hayes (7) figured that of the American *Serica* species.

The epipharynx (Fig. 4 J, K, L) is the ental side of the labrum and is separated from the clypeus by two chitinous plates, known as the tormae (*trm.*). Situated between these plates is the proximal sensory area (*psa.*) which is bordered with non-articulate setae and bears Ritterschaus' sense cone and chitinous plate (*cp.*).

Distal to this area is a space surrounded by inwardly pointing articulate setae which are considerably less in number in *Serica* than in the other two. On the distal border of this space are the chitinous pegs (*ctp.*) and the distal sensory area (*dsa.*). *Serica* again differs by having only a very few of these pegs and a serrated chitinous plate running transverso-longitudinally. On the distal border of the epipharynx there are a number of long articulate setae, with smaller setae round the lateral borders. At the base of these smaller setae are the lateral striae (*lat.str.*), and *Amphimallus* differs from the other two in having in addition two lines of submarginal striae (*sm.str.*) situated in a position slightly lateral to the distal sensory area.

(4) *Mandibles*

The mandibles (Fig. 3 A, B, C) of all three species are very similar. They are the most heavily chitinised part of the head and have three masticating areas, namely the cutting area or dentes (*dent.*) at the distal extremity, the scissorial area (*sca.*), and the molar area (*ma.*) at the proximal end. This molar area, which in mastication probably works against the hypopharyngeal chitinisation, shows a very marked asymmetry between the right and left mandibles, that of the left being larger at the distal end than that of the right mandible. Along the proximal edge of this area is a setaceous portion known as the acia (*ac.*). The caudal side of the mandibles contains the "light area" (*lta.*), which varies in size in each of the three species; on the edge of this is the stridulating area (*sta.*) described by Schiodte (12). In *Melolontha* this consists of a granulated area made up of a number of small pits arranged like the cells of a honeycomb; in this species it is on the distal border of the light area.

In *Amphimallus* and *Serica* this granulation is very minute and can only be seen with difficulty even under the microscope; in the former species it covers most of the light area, but is heaviest on the distal border. In the latter, according to Schiodte (12), it is to be found on the proximal border of the light area, but there is a number (about ten) of large pits on the distal border which may be used in stridulating, since in *Serica* the maxillary scraper is long in comparison with those of the other two species.

(5) *Maxillae*

The maxillae (Fig. 3 D, E, F) are of the general masticatory type, with few modifications. The cardo is divided into two parts, the proximal subcardo (*subc.*) and the distal alacardo (*alac.*). The stipes (*stp.*), which is large and at right angles to the cardo, is connected to the latter and the labium by a membrane known as the labacoria. In *Melolontha* the galea (*gal.*) and the lacinia (*lac.*) are fused to form a mala, although the line of fusion is still marked by a depression. In *Amphimallus* these two joints of the maxillae are only fused at the base, while in *Serica* they are practically free. Both the galea and the lacinia bear a number of chitinous spines and articulate bristles used in mastication.

On the caudal side of each maxilla, and situated on the stipes, is the "maxillary scraper", which consists of a row of chitin teeth; this was described by Schiodte (12) as a part of the stridulating organs. In *Melolontha* the teeth are less in number than in *Amphimallus*, but in both cases they are supported by a number of chitinous ridges which branch off from one central ridge. In *Serica*, on the other hand, the teeth are very numerous and are situated on the edge of a chitinous plate, there being no ridges present. The palpifer (*pf.*) is on the lateral side of the maxilla, and bears a three-jointed palp. At the base of the palp and slightly ental to it, there is in *Melolontha* a chitinous peg of unknown function.

Although these stridulating organs have been described for each of the species, there seems to be no positive record of any sound being heard; the present writer has, however, noted a very distinct scraping sound given out by *Serica* larvae.

(6) *Labium-hypopharynx*

The hypopharynx (Fig. 4 G, H, I) is the ental side of the fused glossa and paraglossa, and has been described for *Phyllopertha* and *Geotrupes* by Carpenter (1) in some detail. The mentum and submentum of the labium project forward in an anterior direction, the ligula being borne at right angles to this in a ventral direction. At the base of the ligula is the hypopharyngeal chitinisation, made up of three sclerites, namely the transverse sclerite (*trs.scl.*) and two lateral sclerites (*lat.scl.*) against which Hayes (7) considers the mandibles to work in mastication. The shape of this chitinous area varies in the three species, but on the right side it always bears Carpenter's "toothed projection". On the left side there is a setaceous area, of fairly large size in *Melolontha*, which that writer considers to be the rudiment of the maxillula (*mxl.*).

Distal to this chitinised area is a very dense patch of setae, those in the centre being thick and pointing in a proximal direction except in *Serica* where they radiate out from a central bare space. On the distal border there are long thin setae pointing in a distal direction, with other setae pointing towards the central line between this and the above area. The labial palps, which are three jointed, are borne on the caudal side of the labium, and have slightly flattened tips which bear groups of sensillae.

(7) *Thorax and abdomen*

There is little apparent difference between the segments of the thorax and abdomen (Fig. 1), except that the former bear three pairs of well-developed legs. There is nevertheless some controversy as to the number of abdominal segments, some writers considering them to be nine and others ten in number. It seems more probable that there are ten and that the last two are becoming fused. Each of the segments is divided up on the dorsal side by transverse folds into a number of annulets, except in the case of the prothorax. The meso- and metathorax and the first five abdominal segments consist of three annulets. The middle one of these three is the largest and broadens out on the pleuron, while the cephalic annulet does not usually extend as far round as this. In *Melolontha* and *Amphimallus* the fifth, sixth and seventh segments have two annulets each, while those posterior to this have only one each. In *Serica* the sixth has three, the seventh and eighth two, and the ninth and tenth segments only one annulet each.

On the side of the prothorax in *Melolontha* and *Amphimallus* there is a rather darker and more heavily chitinised patch. The presence of this patch in the larva of *Cetonia aurata* is usually quoted as distinguishing it from that of *Melolontha*. Since it is present in both larvae this character is of little use for identification; the two larvae are however very different in general appearance.

Most of the body is covered with setae; these are of two kinds, long setae, and short spines which are found mostly on the dorsal side of segments two to seven of the abdomen. In *Melolontha* the long setation is very sparse, there being none on the pleuron or after the sixth abdominal segment. *Amphimallus* is similar except that there are no long setae on the dorsal side of the third to sixth abdominal segments. In

Serica there are considerably more of the long setae than in either of the above species both on the thorax and on the abdomen, but the distribution of the small spines is very much less dense. In all three species the legs bear a number of setae and there is a densely setaceous area, known as the radula, on the ventral side of the last abdominal segment. Since this is of great importance for identification purposes it will be treated separately.

There are nine pairs of spiracles (Fig. 4 D, E, F), one pair being on the prothorax while there are pairs on each of the first eight abdominal segments. Details of the minute structure have been worked out by Ritterschaus (10) for some of the Scarabaeidae, and those of the prothoracic spiracles are shown in the accompanying figures for the three species being here described. The spiracles consist mainly of a central bulla (*bl.*) surrounded by a perforated area through which respiration takes place. The abdominal spiracles only differ from these in that the emargination of this area is on the cephalic side instead of the caudal. In *Melolontha* and *Amphimallus* the size of the abdominal spiracles gradually decreases towards the caudal end. In *Serica*, however, they are all of the same size, but are difficult to locate on the abdomen since they are situated on the edge of a fold in the integument.

(8) *Legs*

There are present three pairs of thoracic legs (Fig. 3 G, H, I), and in the species under consideration they are all well developed, but there is nevertheless a difference in their relative sizes. In all cases the first pair is the smallest and the last the largest. In *Melolontha* and *Serica* the second and third pairs are of equal size, but longer than the first pair; but in *Amphimallus* the second pair is intermediate in size between the first and third pairs. The joints of the legs are generally considered to consist of a long coxa, followed by a short trochanter (*troc.*) borne at right angles to it, and a slightly longer femur (*fem.*). The last joint is usually considered to be either the tibia, with the tarsus wanting, or else formed by the tibia and tarsus having fused, since it bears a terminal claw. There is, however, towards the base of this claw and running round it, a small ridge which may represent the junction where the vestigial tarsus has become fused with the claw. This interpretation is rendered more probable by the facts that this basal portion of the claw bears setae, and is considerably larger in comparison with the remainder of the leg in the first instar larva. These facts are illustrated in the accompanying photomicrographs (Figs. 5 and 6).

In the mature larva the size of the claw varies in each species. Those on the first pair of legs are always the largest, while those on the second pair in both *Amphimallus* and *Serica* are of an almost equal size to them. In *Melolontha* they are smaller than the first pair but larger than those on the last pair of legs which bear the smallest claws in each of the species.

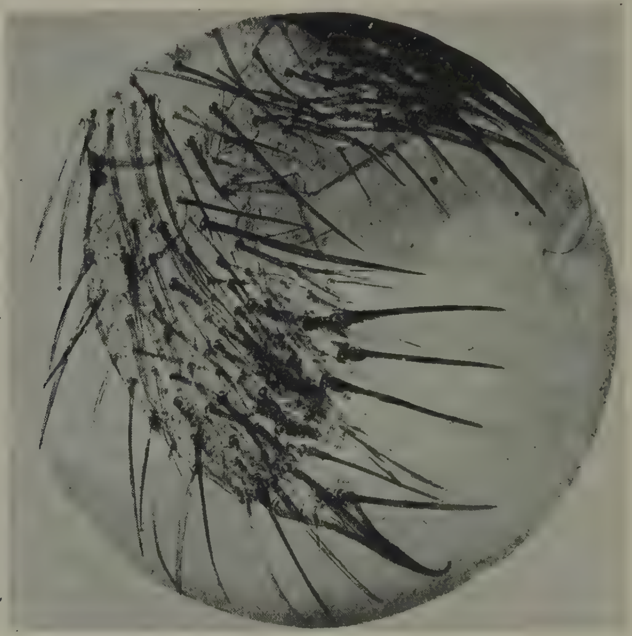

Fig. 5. ***Melolontha.*** **Fourth instar larva, terminal joints of foreleg. × 30.**

(9) *Radula and anal orifice*

The radula (Fig. 4 M, N, O) has been described by a large number of writers, since its study is the easiest means of identification of the larvae in the field. It consists of a densely setaceous area on the ventral side of the last abdominal segment, and is thought to be used for cleaning the mouthparts. As on the rest of the body, the setation consists of long articulate setae (*lst.*) and short spines (*sp.*). In *Melolontha* the short spines are arranged in two rows and point towards the medial line. The rows, which are made up of about twenty-five to thirty spines, converge slightly at both ends and stretch from a point just short of the anal orifice, over three-quarters of the distance to the suture (*sut.*) between the ninth and tenth segments. On either side of these rows are areas covering about one-third of the caudal end of the segment, and which consist of short setae (*hst.*) with the ends turned over like hooks. There are also longer straight setae dorsal to the anal orifice.

In *Amphimallus* the two longitudinal rows of spines which are relatively a little longer than in *Melolontha*, and which are about twelve to fourteen in number, diverge at both ends, especially at the caudal end. The rows are also shorter than in the above species, covering only just over half the distance from the anus to the ninth segment. The areas of hooked setae on either side are triangular in shape, with their apices reaching as far as the cephalic end of the rows of spines, and their bases on a ridge just ventral to the anal orifice. In this species, as in *Melolontha*, there are long straight setae lateral to these areas but also on the dorsal side of the anus.

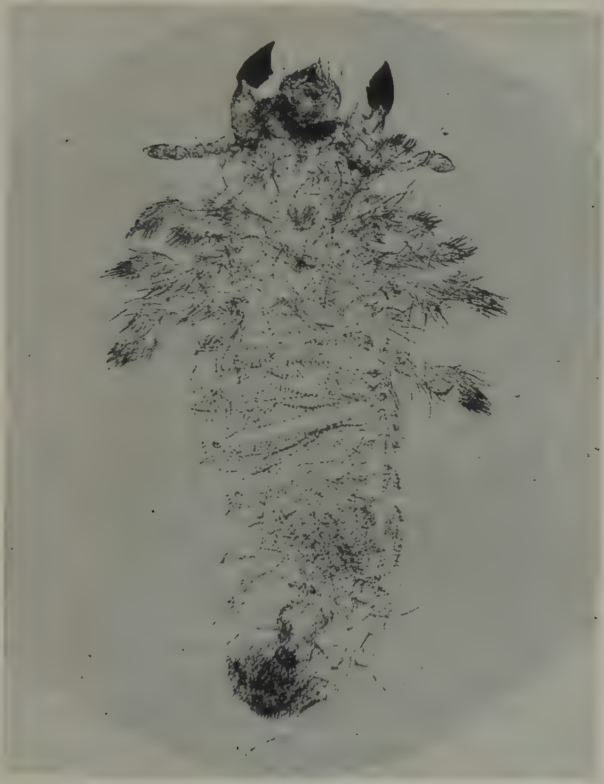

Fig. 6. *Amphimallus*. Newly hatched larva. × 15.

In *Serica* there is only one row of spines which point in a caudal direction, and this runs along the ventral sector of a ridge which encircles the anal orifice. The two areas of short setae, which in this case are not hooked, are triangular in shape as in *Amphimallus*, but much smaller in extent. There is also an area of small setae inside the ridge which surrounds the anal orifice; the whole remainder of the caudal half of this segment is covered with long straight setae. In *Serica* the suture between the ninth and tenth segments is much more marked than in the other two species.

The anal orifice (*ao.*) differs considerably in each of the three species. That of *Melolontha* is terminal and consists of a transverse slit widening in the centre, with a dorsal projecting lobe. *Amphimallus* has an anus surrounded by three lobes, thus forming an orifice triangular in shape and caudo-ventral in position. The opening of the anus is however in the transverse slits, that running longitudinally being only a depression and shorter than the other two. *Serica* has an anal orifice of the same shape as this, except that the longest and most open slit is the longitudinal one; also it is more terminal in position.

C. PUPAL STAGE

The pupae of the Scarabaeoidea do not seem to have received as much attention in the past as the larvae. Although Schiodte (11) gives notes on the pupae of the three genera in question, he does not figure any of the species common in this country. The following comparative descriptions will therefore be given in some detail.

In general form the pupae of the Melolonthinae (Fig. 7) are of the exarate or free type; that is the legs, wings and appendages of the head are without secondary attachment, as is found in the obtect pupae, such as those of the Lepidoptera. The pupae are covered with a thin glossy integument, creamy white in colour; the eyes (*ce.*), spiracles and tips of the anal cerci (*cer.*) are however pigmented a dark brown. The body as a whole is slightly curved in a ventral direction, although the abdomen is capable of movement in a dorsoventral plane.

The head, which is in a position ventral to the pronotum (*pn.*), faces caudoventrally. The appendages of the head can be clearly recognised and are roughly of the adult form; thus it is possible to foretell the sex of the specimen by examining the length of the antennae (*ant.*); in Fig. 7 C is a *Melolontha* female, while males are figured for the other two species. The thorax is less visible from the ventral side, which is figured, than from the dorsal, where it is divided into three sclerites. The first of these,

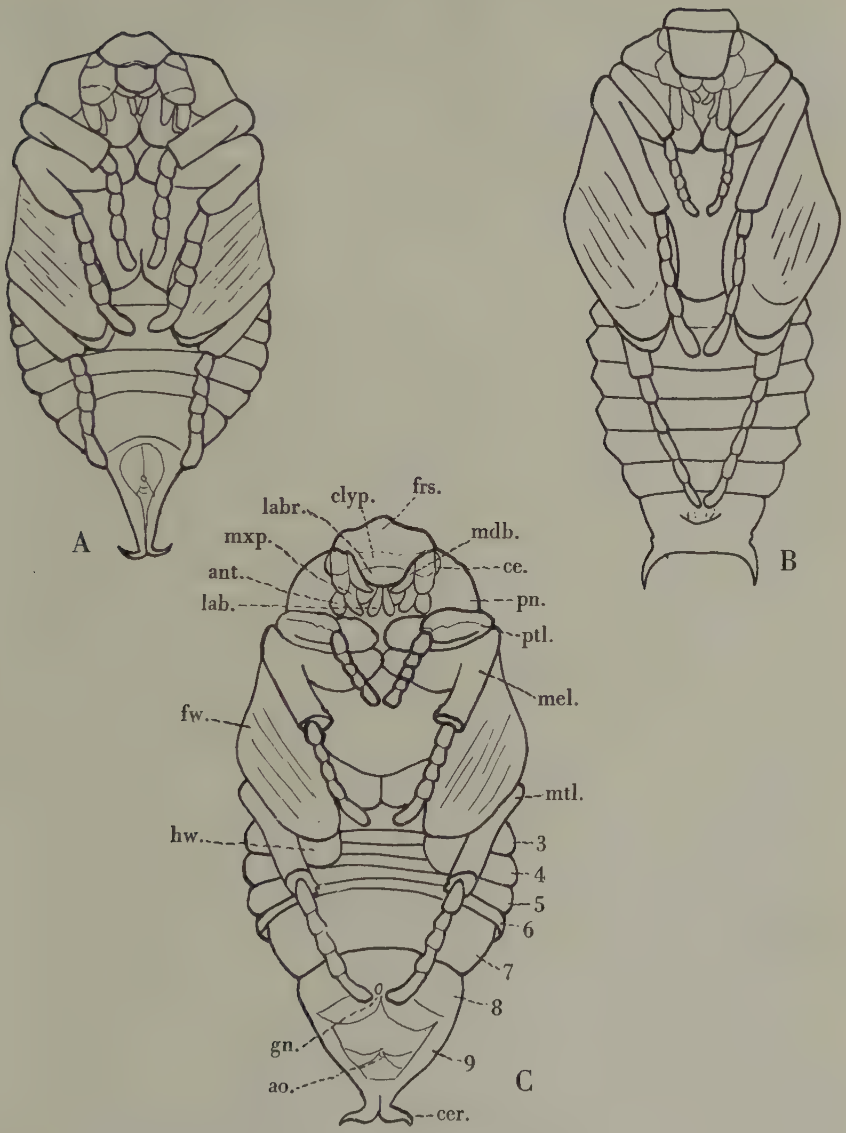
A
B
labr.
clyp.
frs.
mxp.
mdb.
ant.
ce.
lab.
pn.
ptl.
mel.
fw.
mtl.
hw.
3
4
5
6
7
8
gn.
9
ao.
cer.
C

Fig. 7. Pupal stage. Ventral aspect of pupa: A, *Amphimallus* (×3¼); B, *Serica* (×6½); C, *Melolontha* (×3¼). *ant.* antenna; *ao.* anal orifice; *ce.* compound eye; *cer.* cerci; *clyp.* clypeus; *frs.* frons; *fw.* forewing; *gn.* genitalia; *hw.* hindwing; *lab.* labium; *labr.* labrum; *mdb.* mandible; *mel.* mesothoracic leg; *mtl.* metathoracic leg; *mxp.* maxillary palp; *pn*, pronotum; *ptl.* prothoracic leg; 3–9, abdominal segments.

the pronotum (*pn.*), is broadest in the middle, the caudal angles being rounded except in *Melolontha* where they project strongly to the posterior as in the adult stage of this species. The mesonotum is small and rather of the shape of the adult scutellum but broader in *Amphimallus* and *Melolontha*, the apex of this sclerite being transversely rugose in these two species. The third and remaining sclerite of the thorax, the metanotum, is broad and anteriorly subangulate where it meets the apex of the mesonotum.

The legs (*ptl.*, *mel.*, *mtl.*), which in *Serica* are proportionately longer than those of the other two species, are of the adult form, having a similar number of joints. The division of the tarsi are quite visible but there is no claw, the last joint of each leg being similar to the tarsi but with a rounded convex end. The wings are small, the hind-wings (*hw.*) being only slightly larger than the elytra (*fw.*); in general shape they are like elongated, flattened sacs, the apices of which are rounded in the fore-wing and pointed in the hind-wing. The folded adult hind-wing may be seen through the integument in the process of formation, and also the lines and striae of the adult elytra can be just distinguished on the pupal fore-wing. The thoracic spiracles are hidden by the wings except for the first pair which are just visible. These and the first four pairs of abdominal spiracles are large and rounded and produced into a short tube; the remaining spiracles are all smaller.

The abdomen is divided into nine segments (3–9), being broad and rounded at the anterior end, but tapering towards the posterior. The first of these segments in *Melolontha* and *Serica* is raised slightly across the middle in a transverse ridge; in *Amphimallus*, on the other hand, this segment is flattened. The next five segments are strongly convex and rounded, except in *Serica* where the dorsal sides are raised into sharp transverse ridges running across the middle of each segment. The remaining segments of the abdomen are longer and taper gradually towards the caudal end. In *Melolontha* and to a lesser extent in *Amphimallus* the sixth segment is concave posteriorly, the seventh being fitted into it.

In *Amphimallus* and *Melolontha* on the dorsal side of the abdomen between segments four and five, and segments five and six, there is a small pigmented lunate ridge posterior to the middle margin which contains two raised bosses; these appear to be wanting in *Serica*.

On the ventral side of the eighth segment the developing adult genitalia may be seen, while on the ninth is the rudiment of the larval anus. This ninth segment also bears a pair of cerci which differ widely in the three species. In *Melolontha* they are small and fleshy, they and the whole of this segment being slightly and irregularly rugose. Being connate and starting in a posterior direction, they pass outwards laterally, and then at the apex turn towards the posterior again. In *Amphimallus* they are again small and connate, but are horny and sharper than the above; also the apices do not turn towards the posterior but continue in a lateral direction. The cerci of *Serica* differ in being widely separated at their bases; they are relatively larger than in either of the above species, and start in a lateral direction, turning towards the posterior at the apex. The cerci of these three species thus form a ready means for their identification.

In size specimens of each of the three species vary little from their mean, although that figured for *Melolontha* is rather below the average. Thus the length for this species from the base of the anal cerci to the frons is 27 mm., for *Amphimallus* 21 mm., and for *Serica* 10 mm.

SUMMARY

From the foregoing morphological descriptions of the main genera of the British Melolonthinae, it will be seen how much more closely allied *Amphimallus* is to *Melolontha* than is *Serica*. In nearly all details where the former species differs only slightly from *Melolontha*, *Serica* is found to differ more widely. This therefore gives support to the practice of separating this subfamily into the two tribes of *Sericini* and *Melolonthini*.

Since Scarabaeid larvae other than those of the Melolonthinae are also found damaging crops in the British Isles, a table for the rough identification of the most common genera is appended. All species of the Scarabaeoidea are not however included, and since genera such as *Geotrupes* and *Aphodius* may be found at the roots of crops, confusion may occasionally arise in their identification, although they are not known to cause damage to plants. In such cases therefore reference should be made to the foregoing descriptions for exact identification.

1. (2) Body club-shaped at caudal end, antennae long with joints of unequal length .... **3.**
2. (1) Body thickest at middle, antennae short with joints of equal length .... **Cetonia.**
3. (4) Spines of radula arranged in two longitudinal rows .... **5.**
4. (3) Spines of radula arranged in one transverse row .... **Serica.**
5. (6) Longitudinal rows of spines on radula parallel or slightly converging at ends .... **7.**
6. (5) Longitudinal rows of spines on radula diverging especially at caudal end...... **Amphimallus.**
7. (8) Caudal aspect of mandibles with stridulating area made up of small pits ... **Melolontha.**
8. (7) Caudal aspect of mandibles with stridulating area made up of transverse file-like ridges .... **Phyllopertha.**

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THE DISTRIBUTION AND SAMPLING OF INSECT POPULATIONS IN THE FIELD WITH SPECIAL REFERENCE TO THE AMERICAN BOLLWORM, *HELIOTHIS OBSOLETA* FABR.

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(With 4 Text-figures)

A MAIN aspect of insect pest control work at Barberton is based on observations of fluctuations in insect population throughout the year. Records are taken intensively in selected areas where all the crop and weed situations come under review.

In the case of the bollworm moths the course of their activity is ascertained by egg counts on samples of plants and the incidence of larvae is studied. The results of previous investigations (Parsons and Ullyett (7)) showed that egg-laying cannot be considered to occur generally over areas of food plants according to normal expectations. Their results indicated that the moth has preferential instincts for the more well-grown and succulent portions, especially where heavy fruiting, good cover and shade prevail. The intensity of egg-laying, as regarded on a basis of unit areas, has been taken as a criterion of bollworm incidences. Hence it has been necessary to adopt suitable methods for the sampling and analysis of data. The primary considerations were, what is the nature of the distribution of egg-laying and, secondly, how may it be sampled?

The present paper reports the results of recent investigations on these subjects with regard to the American bollworm, *Heliothis obsoleta*. The methods and technique described are, however, equally applicable to the red bollworm, *Diparopsis castanea*, and field studies of other insect pests.

PRECISION DATA

In a recent paper Kalamkar (4) demonstrated the utility of the precision type of experiment to ascertain the variation normally present in a wheat plot. His results showed very clearly the important points to be considered when sampling wheat plots and demonstrated an optimum method for such sampling. In the present work the same type of experiment was designed by the author to find the variation in oviposition within a field of drill-planted maize. No attempt is made in the present paper to fit various sampling methods as done by Kalamkar, although the data could have been used for this purpose. Maize is particularly attractive to the American bollworm and oviposition is relatively extensive.

Material

Parsons and Ullyett (7) showed that maize becomes attractive to the moth when the plants are in tassel. Accordingly a field of maize which was just coming into the attractive stage was selected for the site of the experiment and a plot was taken within the field at random. The dimensions of the selected plot were 23 by 24 yards or approximately one-eighth of an acre. The maize was planted a yard between rows, so the plot

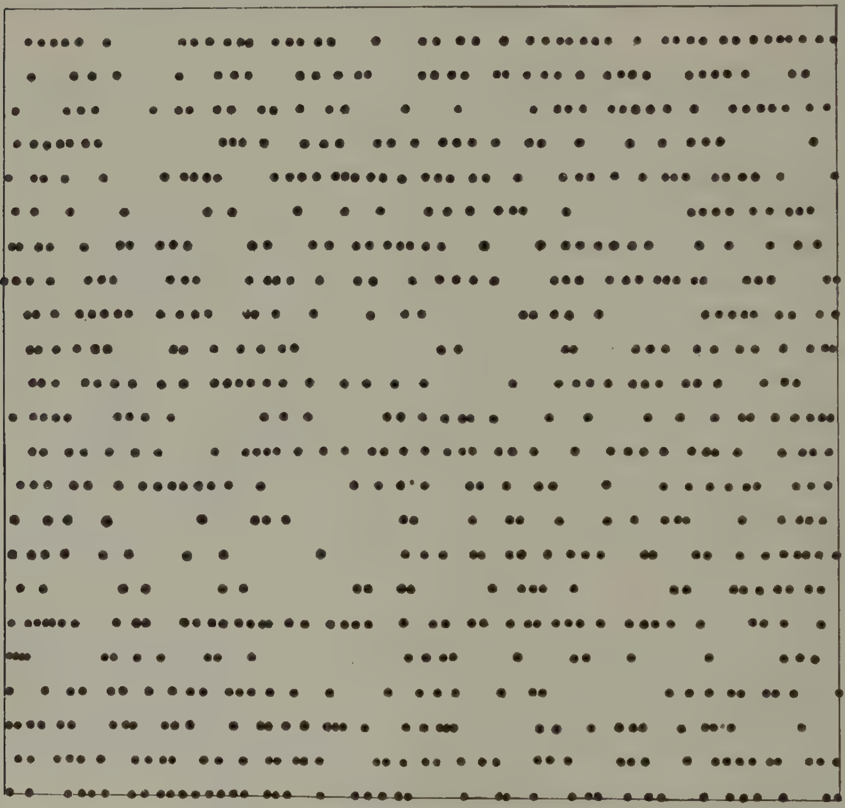

Fig. 1. Plant position in experimental plot.

contained twenty-three rows 24 yards long. For the purpose of subsequent analysis the position of each plant was measured and a complete diagram of the plant population within the plot was secured (Fig. 1). The experiment consisted of counting the eggs on every plant on two occasions with an interval of 1 week between the counts. The eggs of the moth are very minute, but the work was carried out by specially trained natives under white supervision with constant checking. The nature of the work makes a small error unavoidable, but this would certainly be no more than usually occurs in the course of the ordinary routine sampling. An individual count was conducted within the space of 1 day, and the eggs counted were crushed so that there could be no possibility of the second count being in error through the addition of old eggs. The egg counts on both occasions are given in Appendices I and II.

Nature of distribution

The histograms in Fig. 2 represent the frequency of plants in the different egg per plant classes. The distribution for both counts was very

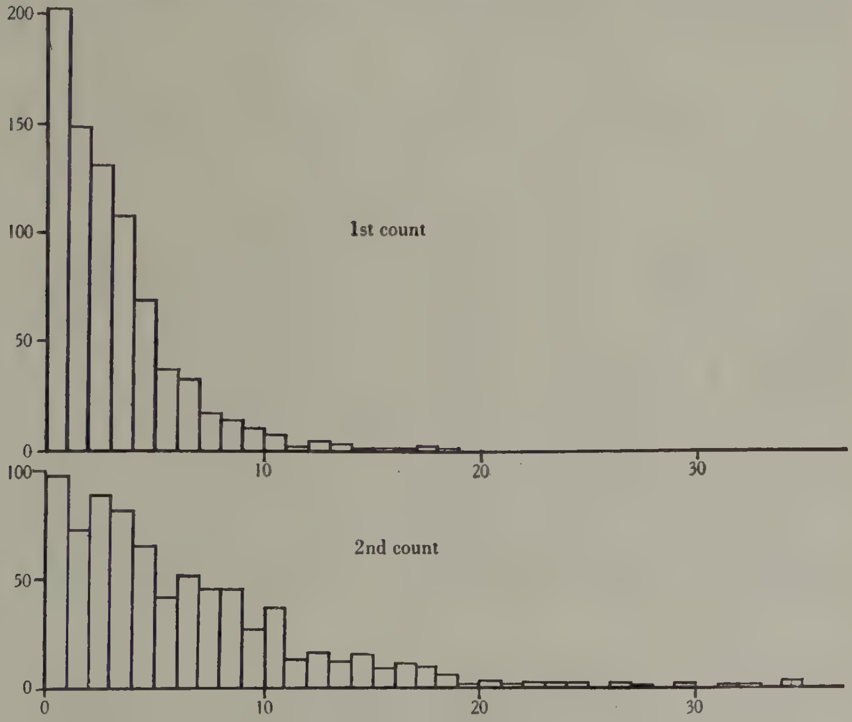

Fig. 2. Distribution of American bollworm eggs on single plants of maize.

similar. The biological aspect of the enquiry, as well as the nature of the distribution curves, suggested a Poisson series, but all attempts to fit such were unsuccessful. This might have been accounted for by the drawn-out tail of the curves where a small number of plants with high egg counts were recorded. Also the minuteness of the eggs and their often hidden position on the plant introduced a considerable error into the zero and few egg per plant classes. Such error would preclude any satisfactory fit. Fits with Poisson series were also tested, using the eggs counted on yard lengths of the rows. Previous measurements made it possible to allocate the plants to their respective yard lengths. There were twenty-three rows each 24 yards long, so that 552-yard length figures were obtained. These figures were now on the basis of area, and since generally two or more plants were in a yard length the grouping would lessen the individual plant error associated with the counting. The nature of the distribution was the same as in the previous grouping, and no fit with a Poisson series was obtained. It is interesting to note that when the egg per yard length classes were grouped and these groups were taken as degrees of intensity of laying 0 to 7, very close fits with a Poisson distribution were realised.

Correlation of egg counts on the same yard length on two occasions

It has been noted (Parsons(6)) that the moth shows definite preferential tendencies to oviposit on certain portions of the crop. *A priori* it was to be expected that over a short period of time, as between the two counts taken in this experiment, that the crop should retain its inherent differences in growth and attractiveness. If this were so then the two counts on the same yard lengths should be positively correlated. A correlation trial was worked out between the first count per yard and the second count on the same yard. (All yard lengths having no plants in them were removed from the analysis.) A significant correlation coefficient was found of +0·3348 with 454 degrees of freedom. This was highly significant at the 0·01 level, and the validity of the correlation was established. A similar correlation on egg counts *per plant* on the two occasions also showed a positive correlation between the two counts. It was therefore definitely established that the same portions of the crop tended to retain their relative attraction to the bollworm moth over short periods of time.

Effect of subdivision of the area on the sampling error

The area of the plot used for the precision experiment was only about one-eighth of an acre and was already smaller than the smallest unit taken in the course of routine sampling. To see whether the sampling error was materially reduced by subdividing the area of the plot the following analysis was compiled:

Table I

Analysis of variance of subplots

Size yards	Between		Within		$\frac{1}{2}\log_e$ diff.
	D.F.	Mean square	D.F.	Mean square	
23 × 12 (2)	1	0·737	550	14·814	−1·500
11½ × 24 (2)	1	155·324	550	14·678	1·179
5¾ × 24 (4)	3	21·874	548	14·748	0·1969
11½ × 12 (4)	3	26·937	548	14·720	0·3017
5¾ × 6 (8)	7	21·414	544	14·701	0·1880

Testing the difference of the mean square for the variation within over the variation between the subplots showed that the limit for z was never reached except in the second entry of the table above. In this case the plot had been divided into two. An equal division in the other direction, as well as smaller divisions, in no instance gave a significant difference. The main evidence was that subdivision of the area would not reduce the sampling error.

Variation between and within rows

Analysis on figures from 3-yard units. All the previous analysis has been carried out using the figures from single yard lengths as the basis for comparison of data. The distribution of these was definitely skew, and the use of Fisher's z test on such might be subject to serious criticism. The distribution of single yard length figures are definitely non-normal, therefore the subsequent analysis was carried out on the egg and plant

Table II

Analysis of variance of egg totals on 3-yard lengths

	D.F.	Sum of squares	Mean square	$\frac{1}{2}\log_e$
First count:				
Between rows	22	1811·973	82·36	1·0543
Within rows	161	9890·005	61·42	0·9076
Total	183	11701·978	—	—
Second count:				
Between rows	22	12731·685	578·73	0·8778
Within rows	161	36269·875	225·279	0·4061
Total	183	49001·560	—	

totals on 3-yard length units. The distribution of these was found to be approximately normal and could be considered within the limits of skewness to allow the use of the z test. With 3-yard units there were twenty-three rows having 8 units each, which gave a total of 184 observations (*vide* Appendices III and IV). Analysis of the variation between and within rows is shown in Table II.

The value of z representing the difference in variation of between rows over within rows was 0·15 and 0·47 for the first and second counts respectively[1]. The 5 per cent. limit for z was 0·21 and the 1 per cent. limit 0·31. In the first count the 5 per cent. level was not quite reached, although in the second the variation between rows was significantly greater than within rows. This result is similar to that obtained by Kalamkar(4) for wheat. The main factor that might have brought about this difference was stand, and the analysis for stand variation was obtained (*vide* Appendix V).

Table III

Analysis of variance for plants per 3-yard figures

	D.F.	Sum of squares	Mean square	$\frac{1}{2}\log_e$
Between rows	22	111·56	5·071	0·8118
Within rows	161	375·75	2·334	0·4238
Total	183	487·31	—	—
				$z=0·3880$

The 1 per cent. limit for z is 0·31, and therefore the variation for plant number (for 3-yard length figures) is significantly greater between rows than the variation within the rows. This indicated a possible relationship between plant number and eggs. Whether the greater variation between rows for the egg counts was due to this difference in stand had to be examined. The greater difference in stand between rows might be accounted for by factors associated with the method of drill planting.

Correlation of plant number and eggs per yard

Since both the egg counts and the plant numbers on the 3-yard lengths disclosed a greater variation between the rows it was expected that there would be a strong correlation between the two. The correlation coefficient between the eggs counted per yard on the first occasion and the number of plants in the same yard was +0·47. For the second count this correlation with the plant number was +0·564 with 550 degrees of freedom. These were highly significant. The regression coefficient for the first count was 2·2, indicating an increase of eggs of this amount for each additional plant over the mean number per yard. For the second count the regression coefficient was 5·1. The relationship between plant number and eggs recorded was clearly established.

---

[1] The distribution of the 3-yard length figures, although approximating to the normal, still have a decided skewness. The z test as applied to the figures may not have been strictly valid, although it has been demonstrated (Eden and Yates(1)) that the test may be correctly applied to distributions of a non-normal form of a slight skewness. As a check on the figures obtained from the 3-yard length totals the same, analysis was carried out on the square roots of these figures. The distribution of these square root values was apparently normal, and the value of z for the greater variation of between rows over within rows was 0·17 and 0·45 for the first and second count respectively. This close agreement demonstrates the validity of the analysis of the 3-yard totals.

Co-variance of plants and egg counts per 3-yard lengths

In view of the correlation between these two factors, as well as the greater variation observed between than within rows, it was desired to see whether eliminating the effect of the stand would eliminate the latter difference. If this difference were accountable for by differences in plant number, it should be possible to eliminate the effect of the latter by the analysis of co-variance. In the present example, assuming that the variations (x) of plant number from their general mean were related to the corresponding variations (y) of egg numbers from their mean for the same 3-yard length by a linear regression, the coefficient (b) was given by the ratio $\frac{\mathrm{Cov}_{xy}}{V_x}$, where Cov_{xy} was the co-variance of the plant number and egg count, and V_x was the variance of the plant number figures. Using this coefficient, the egg-count figures could be adjusted for the plant number in the individual 3-yard lengths and comparison of these adjusted figures would become comparisons of the quantity $(y-bx)$. The preliminary analysis of the figures gave:

Table IV

Analysis of sums and products. Deviations for plants (x) and eggs (y) per 3-yard lengths

	D.F.	x^2	xy	y^2
First count:				
Between rows	22	111·56	349·046	1811·973
Within rows	161	375·75	1184·500	9890·005
Total	183	487·31	1533·546	11701·978
Second count:				
Between rows	22	111·56	701·806	12731·685
Within rows	161	375·75	2001·249	36269·875
Total	183	487·31	2703·055	49001·560

The relationship, expressed by the regression of eggs on plants (that is x on y), was determined by the value 1184·500/375·75 for the first count and 2001·249/375·75 for the second. These values represented the respective amounts for the two counts that had to be deducted from any value y for each plant by which the corresponding x was in excess of the mean.

As stated above, the adjusted egg numbers were represented by the quantity $(y-bx)$ and expanding:

$$(y-bx)^2=S(y^2)-2bS(xy)+b^2S(x^2).$$

Accordingly the adjusted values for the egg counts were obtained by multiplying across in the above table x^2 by b^2, xy by $-2b$ and y^2 by unity and summating.

Table V

Analysis of adjusted number of eggs per 3-yard lengths

	D.F.	Sum of squares	Mean square	$\frac{1}{2}\log_e$
First count:				
Between rows	22	719·9487	32·725	0·5928
Within rows	160	6156·0308	38·475	0·6737
			z diff.	0·0809
Second count:				
Between rows	22	8420·5892	382·754	0·6712
Within rows	160	25611·1979	160·070	0·2352
			z diff.	0·4360

The adjusted figures were obtained by using the coefficient obtained from the co-variance and variance within rows. According to Yates (9) a correction, which is in the nature of a subtraction, has to be applied to the adjusted variance for between rows. In this instance the subtraction was negligible and did not modify the result. Adjusting the figures of the egg counts for stand in the first count now gave a higher variation within rows than between rows. The previous value for z of 0·15 was probably due entirely to effect of stand variation.

In the second count the z value of 0·4360 was still highly significant and practically the same as that obtained on the unadjusted egg figures. The fact that the greater variation between rows in the first egg counts was entirely accounted for by the differences in plant number as well as the high correlation between egg and plant number showed clearly that plant number had important bearing on egg-laying. Yet in the second count the same differences in plant number had little effect in diminishing between-row variation. Some other factor must have influenced the laying in the second count. The only differences in the experimental plot conditions for the second count were first that the plants were 1 week older, and secondly that the egg-laying was double the intensity of the first. In the first count the maize was just coming into tassel, whereas in the second the plants were in full tassel. In this latter condition secondary effects of stand such as size of plant and earlier tasselling (due to lack of competition from adjacent plants in portions of low stand) would influence the egg-laying considerably. In some cases very early plants would be losing their attraction to the moth. However, in both counts it was striking that differences from row to row were very considerable, and it follows that in any method of sampling care should be exercised to provide that as many rows as possible are sampled rather than that sampling should be confined to a few rows only.

Distribution of means of yard lengths

In the analysis of the sampling data in the next section the mean of the eggs laid on a number of random yard lengths are examined statistically. To test the validity of assuming these means to be distributed normally the number of eggs laid on all the yard lengths in the precision experiment were completely randomised and withdrawn grouped in fours. The sums of the 4-yard lengths obtained in this way were distributed as depicted in the histogram (Fig. 3).

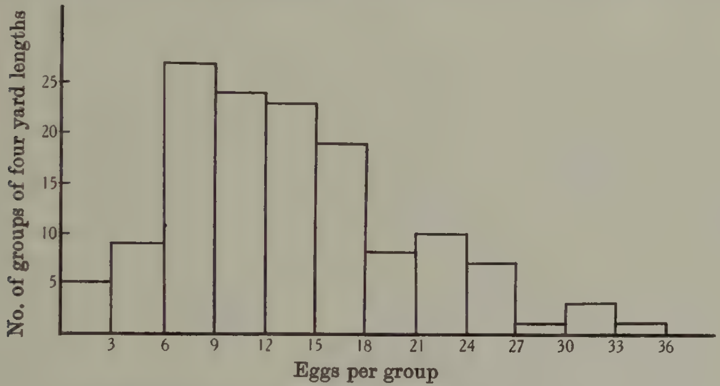

Fig. 3. Histogram.—Total eggs on groups of four random yard lengths.

Testing the normality of this data after Fisher ((2), p. 52) the value for the parameters γ_1 and γ_2 were $0{\cdot}393 \pm 0{\cdot}21$, and $-0{\cdot}07 \pm 0{\cdot}43$ respectively. Thus γ_1 exceeded its standard error, and since this parameter is essentially a test for asymmetry the fact that it was positive suggested, but did not prove, that there was a tendency for low egg counts and very high egg counts to be more frequent than moderately high or very low counts. This value of γ_1 was very small, and it followed that the asymmetry was not at all marked, and therefore the distribution of 4-yard lengths could be treated as normal data. The other parameter γ_2, which measured any symmetrical departure from the normal where the apex and tail would be developed at the expense of the intermediate portions of the curve, was negative and less than its own standard error and is therefore insignificant. Earlier data were tested in the same way and were found to give complete agreement with the above, namely that the sums of 4-yard lengths were distributed according to normal expectations. It is on these results that the sampling technique, which is discussed later, was evolved.

Sampling considerations

From the figures given it has been shown that the variation in the number of eggs laid per plant was very great. The largest number of plants fell in the zero class, while several plants had very large numbers of eggs laid on them as compared with the general mean. Consequently any method of sampling must make provision for this diversity, for if either end of the distribution only were sampled a very incorrect idea of the egg-laying would result. Also sampling an egg-laying differs from sampling for crop measurements, in so far as the eggs represent insect activity which has been shown to be responsive to particular phases of plant development with discrimination between small differences in health and environment of the food plant. For these reasons any qualitative samples selected from the crop might represent very inadequately the activity of the moth over the whole field. At the same time if a field has sections which are obviously far in advance of the rest then it is much better that the field be divided into smaller subsections each of which is sampled independently, and the infestation over the whole field be obtained from compiling the several data so secured. Having decided what the area to be sampled separately should be, then a completely random set of samples must be scattered within it. From these it is necessary to obtain some estimate of the error of sampling. It has been shown that if the total of four separate yard lengths were taken these could be considered as having a normal distribution, and so the standard error from such totals could be correctly estimated according to accepted methods applicable to such data.

SAMPLING DATA

From the results of the preceding section the authenticity of considering the totals of four or more yards counts as having a normal distribution has been established. All the sampling analysis to be described was based on this finding. Similarly the sampling units themselves were always taken on an area basis, so that the figures obtained could be expressed in terms of eggs per acre, thus providing a picture of the intensity of infestation.

It has been mentioned that the system of survey of the American bollworm activity is carried out on selected areas wherein all the crops are sampled. Owing to the wide expanse of the crops it was desired to find a sampling method which gave the required standard of efficiency and at the same time necessitated taking only a small percentage sample of the whole. A method was adopted at Barberton based on that devised by the Statistical Department of the Rothamsted Experimental Station for obtaining estimates of the yields of fields of commercial wheat. Several alterations were, however, introduced in order to comply with the requirements of the problem in hand.

Technique

The sampling procedure adopted provided for four series of sampling units, and it was from the sums of the pairs of related series that the error was estimated. A restriction was imposed on the method. In the first place the samples in each series were made to fall at intervals of one-fifth of the length of the field so that one sample in each of the four series fell in one-fifth. Thus four samples were taken from each fifth of the field. Secondly, in order to get lateral scatter of the samples a number of rows were stepped over between each sampling point. This is an important consideration. The precision experiment has shown that the variation between rows is greater than within rows. Also since the moth has preferential instincts to lay on certain portions, then restrictions which make for a scatter over the field must tend to counteract any chance of obtaining a biased group of samples.

Each series of sampling units had either four or five units in it, the size of these varying according to the size of the field sampled. The unit sample is based on a yard length on a single row where the between-row spacing is 1 yard; this gives a sample of 1 square yard. Where more than 1 square yard sample is necessary then 1-yard lengths in contiguous rows are taken.

A sample lay-out is given in Fig. 4, which represents a field of cotton planted a yard by a yard. The size was about 200 by 100 yards. Using *AB* as the starting line, two series of sampling units was marked out walking towards *CD*, and the other two series on walking from *CD* back to *AB*. In sampling the field a starting point was found on *AB* by obtaining a random number from Tippett's Tables(8) which was no greater than the length *AB*. This located the starting row to be 27 yards along *AB*. The length of the field was estimated to the nearest 50 yards, and the number (200 yards) was divided by five, giving 40. The dotted lines in the figure represent the division of the field into five parts. To ascertain the position of the first units of the first two series of sampling units two random numbers, no greater than 40, were obtained. These were 10 and 25. The first unit sample of series A was therefore 10 yards up the starting row, and consisted in this instance of a 2 square yard sample represented by the plants in a yard length in two contiguous rows. The first sample of series B was then located by stepping over ten rows to the left and walking up the row until 25 yards from the

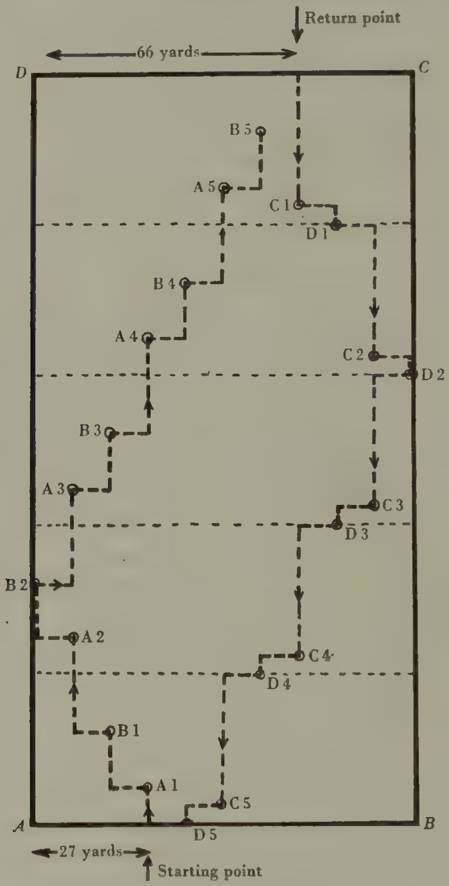
Return point
66 yards
D
C
B 5
A 5
C 1
D 1
B 4
A 4
C 2
D 2
B 3
A 3
C 3
D 3
B 2
A 2
C 4
D 4
B 1
A 1
C 5
D 5
A
B
27 yards
Starting point

Fig. 4. Sampling method.

base line *AB*. In effect this was 15 yards up the field from the first sampling part of series A. (The moving over of ten rows to the left was purely an arbitrary length adopted to afford a better scatter of the samples.) The second sampling point in series A was then found by again moving over ten rows to the left and walking towards *CD* until 40 yards from the first sample of the same series (that is 25 yards from the first sample point of series B). The second sample in series B was found by moving over ten rows to the left and walking towards *CD* for 15 yards (that is, 40 yards from the previous sample of the sample series). In all, five samples were marked out in each series, and since each sample within a series was 40 yards removed from the other this resulted in one sample of each series falling within the five subdivisions of the field. If, as in this example, the course of sampling brings one to the edge of the field, then instead of moving 10 yards to the left between each sampling point the same amount was in future stepped to the right.

For the other two series, C and D, the above procedure was repeated starting from *CD*. A new random starting row along *CD* and two new random numbers up to 40 were obtained. The starting row was 66 yards from *D* and the two numbers were 35 and 40.

The sampling method has the defect that all parts of the field have not the same probability of being sampled. Indeed there are certain parts of the field which cannot be sampled at all by series A and B, and other corresponding parts which cannot be sampled by series C and D. The defect could be avoided in rectangular fields by starting again at the opposite side of the field when the edge is reached, and always proceeding diagonally *in the same direction* across the field. Inasmuch as in insect survey work fields of irregular shape have to be sampled, however, the adoption of a procedure which is theoretically perfect for rectangular fields is not of great importance.

It may be also noted that series A and series B come from two sets of rows which are equally spaced. It might have been argued that two random numbers adding up to 20, *e.g.* 7 and 13, to give the alternate number of rows stepped over should have been taken. This was not adopted, since it would have complicated the locating of sample plants by native recorders in crops where markers are obscured.

The four series gave 20 units, and these being each 2 square yards gave a potential sample of forty plants if the stand were perfect. The area sampled was about 4 acres, thus the sample was about 0·2 per cent. of the field. It will be noted that there were 20 unit samples, each sample had single plants in two contiguous rows, thus, in all, forty rows were sampled. This is in agreement with the findings of the precision experiment.

The four series cannot be regarded as independent. Series A and B are related and so are C and D. The true sampling error is computed from the difference of the mean of A and B from the mean of C and D. In the subsequent analysis the figures presented are the totals of the series A and B, and C and D respectively.

Analysis of results

The land at the Cotton Station, Barberton, has been divided into plots of 2·2 acres, which in some instances have been subdivided by a path into two subplots of 1·1 acres each. In these subplots a sample of four series was taken, each having within it only four units of 1 square yard.

(*a*) *Maize, Cotton Experiment Station, Barberton.*

The figures in Table VI represent the number of American bollworm eggs counted on eight plots on six occasions. Each plot has the total number of eggs recorded on the two sets of series. The period of the records given covers the principal oviposition during tasselation. The intensity of the egg-laying was moderate.

Table VI

Plots

Occasions	Plots								
	1	2	3	4	5	6	7	8	
1	1	0	10	11	24	21	11	5	
	3	2	12	4	14	17	5	6	146
2	16	0	35	35	62	55	31	7	
	18	1	54	23	72	74	10	18	511
3	21	12	102	50	47	71	32	23	
	21	13	37	36	73	46	26	42	652
4	8	7	32	35	49	43	12	10	
	12	5	21	6	41	30	18	15	344
5	20	57	12	8	2	2	14	15	
	56	22	18	8	12	17	17	18	298
6	5	5	4	4	2	1	14	24	
	12	9	6	4	2	4	17	4	117
	193	133	343	224	400	381	207	187	
								Grand total	2068

The efficiency of sampling can be best estimated by a comparison of the within-plot variance due to sampling with the variance between plots. The egg-laying figures were counts taken on the same sample plants on all occasions, and therefore the figures for successive occasions are not really independent. Consequently, when comparing plots over all occasions the errors must be computed from the totals of all occasions, while the errors for comparing variation of plots from occasion to occasion must be obtained by using the sampling and error variance between occasions. Working in terms of a single occasion and a single sampling unit (a single line) the analysis gives:

Table VII

Analysis of variance, maize plots

(In units of number of eggs per line per occasion: mean number = 21·54)

	D.F.	Sum of squares	Mean square	Mean square if whole field had been sampled
Total of all occasions:				
Between plots	7	5930·3	847·19	731·50
Within plots between lines	8	925·5	115·69	—
Within lines between series	16	1250·0	78·13	—
Differences between occasions:				
Between occasions	5	13474·9	2694·98	2561·47
Plots × occasions	35	14209·6	405·99	272·48
Lines × occasions	40	5340·5	133·51	—
Within lines between series	80	3614·0	45·18	—

Percentage sampling standard errors:

For totals of all occasions (per plot) = 14·5 %

For differences between occasions (per plot per occasion) = 38·1 %

The adequacy of the sampling is shown by the last column, which gives the differences between the mean squares between plots, etc., and the corresponding sampling mean squares. These differences are estimates of what the mean squares would have been had the whole of each plot been sampled. (The correction for the fact that the number of sampling units in a plot is finite is here negligible since only 0·2 per cent. of the field was sampled.)

The mean square between plots on all occasions is reduced from 847·19 to 731·50, only a small reduction. Little, therefore, would be gained by increasing the number of lines sampled (with the same number of units per line): doubling the number of lines for instance, would reduce the variance by $\frac{1}{2}$ (115·69) to 789·35. The increase in accuracy by taking double the number of series per line (with the same number of lines) is obtained from the mean square within lines between series the variance being reduced by $\frac{1}{2}$ (78·13) to 808·13. In the case of plots × occasions the reductions, though proportionately larger, are also small. We may, therefore, conclude that the sampling is adequate even for the purpose of comparing plots of the same area. For the purposes of Insect Pest Control Survey, where variation between different fields affects the results, it may be expected to be even more adequate. In fact some reduction of the percentage of sampling might be advantageous. On farms where the averages are large a smaller percentage sample is taken.

(*b*) *Cotton, Cotton Experiment Station.*

The same sampling procedure was used but only a very light infestation was experienced, since the maize "took" the main laying at the beginning of the season. There were never more than two eggs recorded on one plant and the majority gave zero counts. The mean over all occasions was 0·22 egg per plant. On such a light laying a small sample must necessarily have a high error, especially where single eggs occur only on one in four plants. Additionally the errors due to observation on such a light egg-laying assume large proportions and it would be unreasonable to expect a high efficiency under the circumstances. The analysis of variance, in units of a single occasion and a single sampling unit (a line), was as follows:

Table VIII

Analysis of variance, cotton plots

(Eleven plots, eleven occasions : in units of mean number of eggs per line per occasion : mean number = 1·764).

	D.F.	Sum of squares	Mean square	Mean square if whole field had been sampled
Total of all occasions:				
Between plots	10	211·35	21·135	13·887
Within plots between lines	11	79·73	7·248	—
Within lines between series	22	120·64	5·484	—
Differences between occasions:				
Between occasions	10	442·16	44·216	37·710
Plots × occasions	100	650·57	6·506	2·908
Lines × occasions	110	395·77	3·598	—
Within lines between series	220	706·36	3·211	—

Percentage sampling standard errors:

For totals of all occasions (per plot) = 32·5 %

For differences between occasions (per plot per occasion) = 76·1 %

Such low infestations are not required to be known very accurately, and it would appear that here also the sampling is quite adequate.

For maize the period of observation of egg-laying is short and the sampling plants are never changed. With cotton the sample plants are changed occasionally to avoid the effects of continuous handling. This effect on the growth rate of cotton has been observed at this station (Heath (3)). In the case of cotton the sample plants were not changed during the six occasions quoted, as this represented a period of 3 weeks only.

SUMMARY

1. The problem associated with estimation of insect activity is discussed.

2. A precision experiment, undertaken to investigate the nature of the egg-laying of the American bollworm, is described. The nature of the distribution is investigated.

3. Correlation trials between the two counts show that the same portions of the crop retain their relative attraction to the bollworm moth over short periods.

4. The effect of subdivision of the area on the sampling error is analysed.

5. Both egg as well as plant number figures per 3-yard length show a greater variation between than within rows.

6. Correlation and regression coefficients prove a very close relationship between plant number and eggs laid.

7. Eliminating the effect of plant number eliminates this greater variation for the first egg-count figures. In the second count the variation between rows is still significantly greater. The reason for this is discussed.

8. Considerations affecting the sampling technique developed from the precision data are discussed.

9. The sampling method is explained and two examples are given. From the analysis of these the system is shown to sample adequately the fields and fulfills the requirements of the survey.

ACKNOWLEDGMENTS

The necessity of obtaining sampling procedures applicable to field studies of insect populations was put forward by Mr F. S. Parsons at the Second Cotton Conference in London (5). The sampling method was introduced by Mr Parsons and the investigations reported were undertaken at his suggestion. The author desires to thank him for his valuable advice and criticism.

The author also wishes to thank Mr M. F. Rose and Mr O. V. S. Heath, of this Station, for reading the manuscript. Mr Heath also gave many helpful suggestions as to the treatment of the data.

It is desired to acknowledge gratefully the several points suggested by Mr Yates in the statistical presentation of the data, and to thank Dr H. F. Barnes for seeing the paper through the press.

APPENDIX I

Eggs per plant: first count

Row No.																																														Total
1	4	0	3	2	2	1	0	0	5	1	4	0	1	3	1	1	2	0	1	1	2	3	12	1	2	7	0	0	0	1	0	3	0	2	3	5	3	4	0	1	3	4	.	.	.	88
2	0	1	2	1	0	8	1	0	1	5	0	6	0	3	1	0	5	0	0	1	0	0	3	0	6	1	1	2	4	5	0	1	3	0	3	2	4	6	5	0	4	2	0	.	.	87
3	2	3	0	0	3	2	0	2	0	3	0	3	0	1	0	0	4	2	3	5	4	16	8	0	14	3	2	2	4	0	6	1	2	3	4	1	5	.	.	.	.	.	.	.	.	108
4	1	2	0	1	3	4	0	1	4	0	3	1	1	2	1	0	4	2	0	1	2	5	2	3	1	0	2	3	2	5	1	0	.	.	.	.	.	.	.	.	.	.	.	.	.	57
5	3	1	3	3	0	2	1	1	6	0	0	3	2	4	2	1	0	6	0	0	5	0	1	3	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	47
6	1	0	0	0	2	4	3	0	6	4	3	0	13	8	0	17	0	8	2	12	3	0	0	0	2	0	2	0	0	0	2	0	3	0	3	1	2	0	0	4	11	1	3	2	2	124
7	4	0	6	3	0	0	0	2	0	6	1	0	2	0	0	0	4	3	2	0	2	2	6	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	44
8	3	0	4	4	2	5	1	1	2	3	0	1	0	0	1	1	2	2	0	6	3	0	3	3	2	2	1	0	2	0	3	.	.	.	.	.	.	.	.	.	.	.	.	.	.	57
9	2	0	3	1	1	0	1	4	0	0	5	2	3	1	2	0	7	2	0	0	1	6	0	7	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	48
10	0	1	2	2	8	0	0	2	3	5	4	0	7	4	0	0	0	2	3	1	3	3	2	2	6	0	5	8	0	0	8	3	1	.	.	.	.	.	.	.	.	.	.	.	.	85
11	0	0	1	2	0	13	1	3	7	3	1	0	2	1	0	4	1	7	4	4	0	2	1	0	10	6	0	0	5	0	6	6	0	2	9	3	4	0	.	.	.	.	.	.	.	108
12	1	2	0	4	2	4	0	4	10	2	2	8	2	4	2	1	0	1	0	6	0	1	3	5	2	1	1	1	1	1	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	72
13	2	4	6	1	3	3	5	4	3	3	2	0	1	2	3	4	5	3	2	1	4	1	1	0	5	0	6	2	2	3	1	5	2	0	3	.	.	.	.	.	.	.	.	.	.	92
14	8	3	0	7	0	2	3	0	0	1	2	0	4	0	6	4	1	0	1	2	5	0	5	7	4	1	2	3	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	71
15	5	2	3	7	10	4	2	3	1	6	4	2	7	9	6	0	4	2	5	2	3	2	1	2	7	1	8	1	0	0	0	3	5	0	3	.	.	.	.	.	.	.	.	.	.	120
16	9	3	2	5	3	3	1	2	6	1	1	1	0	0	4	3	4	5	2	3	7	4	0	3	4	3	2	1	2	0	3	0	1	2	0	2	4	.	.	.	.	.	.	.	.	96
17	0	0	2	3	5	2	0	6	0	0	0	1	5	5	0	1	8	0	0	1	1	1	4	0	1	0	4	1	2	6	4	0	1	0	.	.	.	.	.	.	.	.	.	.	.	64
18	0	1	3	2	1	0	1	2	4	2	3	1	4	1	1	1	0	0	0	2	4	1	3	3	6	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	46
19	2	3	2	0	0	0	8	1	3	0	0	0	0	0	0	10	4	1	9	0	2	0	0	3	0	2	1	1	4	3	2	2	3	1	0	2	6	5	12	1	0	.	.	.	.	93
20	4	0	0	1	4	3	1	3	4	2	4	3	2	1	2	3	3	2	1	3	1	1	6	2	17	1	2	15	2	4	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	97
21	0	1	0	5	0	4	7	8	0	0	4	1	13	1	0	1	3	3	7	1	1	7	5	11	2	9	0	1	3	3	2	2	3	.	.	.	.	.	.	.	.	.	.	.	.	108
22	2	0	1	6	9	0	0	0	3	2	1	2	4	3	1	3	2	4	1	2	3	3	4	1	3	7	1	9	0	10	5	6	4	4	.	.	.	.	.	.	.	.	.	.	.	106
23	0	4	1	0	0	0	2	1	2	2	3	2	1	1	3	10	1	18	0	1	5	1	0	3	10	6	0	0	4	1	1	0	4	1	0	2	0	2	3	0	0	6	7	8	.	116
																																														1934

APPENDIX II

Eggs per plant: second count

Row No.																																														Total
1	3	7	1	4	2	15	1	0	1	12	2	3	2	10	16	12	5	7	0	8	4	10	7	12	3	7	14	1	5	9	6	0	10	4	5	8	2	5	6	8	6	9	.	.	.	252
2	5	3	8	3	2	10	0	3	16	4	13	3	8	7	5	7	2	1	6	10	14	6	16	2	6	15	8	3	7	3	10	6	20	1	9	3	27	9	13	0	11	2	7	.	.	314
3	34	22	2	8	2	2	0	2	1	13	9	5	3	3	3	0	3	2	1	8	16	17	17	8	34	14	8	17	14	0	15	3	12	10	10	1	18	.	.	.	.	.	.	.	.	337
4	12	0	7	6	16	10	2	2	0	26	3	17	2	10	11	10	21	9	0	2	0	6	3	1	0	1	2	5	9	14	0	6	.	.	.	.	.	.	.	.	.	.	.	.	.	213
5	2	6	1	7	0	6	3	7	9	6	1	0	14	10	3	2	0	15	14	4	1	32	1	17	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	161
6	6	2	7	1	13	10	8	1	16	11	12	11	6	4	23	1	2	4	10	10	2	4	0	9	0	13	5	1	3	11	3	13	2	29	8	14	8	10	6	2	0	12	17	4	10	344
7	15	0	11	8	3	0	8	4	5	9	51	6	1	7	8	0	3	7	4	1	3	9	10	17	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	190
8	4	0	10	22	6	7	8	2	18	2	7	2	3	0	2	0	11	5	34	8	2	2	23	6	3	15	5	1	3	1	14	.	.	.	.	.	.	.	.	.	.	.	.	.	.	226
9	14	3	4	0	2	1	17	9	1	1	7	4	13	0	7	1	4	3	0	2	6	11	1	8	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	119
10	4	1	6	4	8	5	3	11	12	2	9	2	17	8	8	4	2	6	8	1	5	1	3	10	1	0	10	17	8	8	18	0	2	.	.	.	.	.	.	.	.	.	.	.	.	204
11	3	2	3	6	4	29	0	20	19	2	1	0	8	0	2	10	4	8	10	12	1	0	5	3	9	4	0	0	1	2	6	6	0	8	7	6	9	2	.	.	.	.	.	.	.	212
12	4	2	4	10	13	3	4	18	14	5	4	13	9	15	7	8	10	6	3	3	1	0	2	2	0	18	0	20	0	4	11	.	.	.	.	.	.	.	.	.	.	.	.	.	.	213
13	3	1	8	8	3	10	9	5	10	3	0	1	5	1	1	10	4	2	7	0	1	4	3	5	0	0	2	16	6	3	8	0	1	3	0	.	.	.	.	.	.	.	.	.	.	143
14	3	2	4	7	2	3	10	0	1	3	4	0	0	7	3	9	3	14	6	4	1	3	3	13	4	0	7	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	118
15	4	3	7	16	3	2	1	0	6	0	1	12	5	2	12	8	6	8	4	1	2	5	4	11	6	7	1	0	0	1	3	7	8	0	2	.	.	.	.	.	.	.	.	.	.	158
16	7	2	0	8	7	4	3	6	14	3	2	4	3	0	8	7	2	4	10	8	12	0	4	0	7	4	7	8	4	1	0	5	2	2	1	2	0	.	.	.	.	.	.	.	.	161
17	0	0	8	3	12	16	2	10	0	0	2	2	15	5	4	2	3	0	2	0	1	4	6	6	10	15	4	4	3	9	0	10	2	6	.	.	.	.	.	.	.	.	.	.	.	166
18	2	0	4	18	8	2	0	3	5	24	6	3	8	6	10	0	2	7	10	14	9	6	7	10	6	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	170
19	5	3	0	2	0	0	6	4	3	0	4	5	0	0	1	8	5	1	3	6	3	2	1	0	1	1	2	3	1	4	3	0	0	0	4	7	5	1	5	11	0	4	5	.	.	119
20	6	0	2	0	4	7	0	3	10	9	3	6	4	0	1	2	16	3	6	5	0	1	3	2	9	6	3	4	12	3	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	130
21	2	4	1	3	0	4	6	5	7	0	5	3	6	3	1	2	1	4	17	3	0	0	2	3	4	3	0	1	3	2	1	6	18	.	.	.	.	.	.	.	.	.	.	.	.	120
22	6	0	13	8	7	5	2	3	9	2	15	7	5	4	4	1	2	0	5	2	2	3	1	2	6	6	8	12	4	9	5	6	1	4	.	.	.	.	.	.	.	.	.	.	.	169
23	2	9	3	0	4	0	7	10	4	11	0	6	8	10	1	12	5	8	8	9	15	7	5	9	4	7	3	2	0	8	12	9	1	1	5	0	4	6	15	7	6	2	2	13	.	260
																																														4499

APPENDIX III

Eggs per 3-yard lengths: first count

Row No.									Total
1	11	6	9	4	19	10	14	15	88
2	13	6	10	6	10	13	12	17	87
3	2	8	5	4	14	42	17	16	108
4	7	9	3	4	5	10	11	8	57
5	10	0	4	9	2	6	7	9	47
6	1	9	34	39	5	4	9	23	124
7	13	2	7	2	7	2	4	7	44
8	19	6	2	14	3	3	4	6	57
9	7	5	5	6	2	9	0	14	48
10	13	5	9	11	5	4	26	12	85
11	3	27	4	20	13	17	8	16	108
12	13	16	10	9	1	7	11	5	72
13	12	22	5	7	10	12	13	11	92
14	20	6	4	6	0	8	5	22	71
15	31	12	28	4	7	8	19	11	120
16	22	14	8	14	14	10	5	9	96
17	2	10	7	10	11	5	8	11	64
18	7	7	2	11	0	2	5	12	46
19	7	12	0	26	7	11	6	24	93
20	4	8	8	11	11	12	0	43	97
21	10	19	19	3	9	34	4	10	108
22	3	18	13	15	11	17	15	14	106
23	8	11	38	20	4	7	4	24	116
									1934

APPENDIX IV

Eggs per 3-yard lengths: second count

Row No.									Total
1	17	17	29	33	36	42	42	36	252
2	31	36	30	33	45	37	60	42	314
3	34	36	25	14	14	92	68	54	337
4	41	14	29	29	42	17	12	29	213
5	16	0	16	16	14	13	31	55	161
6	16	32	83	27	15	49	71	51	344
7	37	17	66	8	18	5	12	27	190
8	57	29	7	60	25	6	18	24	226
9	23	28	8	17	8	7	2	26	119
10	23	31	13	37	16	6	42	36	204
11	18	70	9	46	18	13	14	24	212
12	40	37	17	49	9	4	22	35	213
13	12	48	7	11	13	13	27	12	143
14	21	18	0	10	0	36	4	29	118
15	35	7	32	14	12	12	25	21	158
16	24	32	22	24	16	26	10	7	161
17	8	33	14	24	8	16	36	27	166
18	32	10	24	33	9	24	15	23	170
19	10	17	6	26	10	8	17	25	119
20	6	13	13	22	22	15	0	39	130
21	14	23	16	4	20	12	4	27	120
22	19	34	37	12	12	30	14	11	169
23	35	39	58	32	5	36	10	45	260
									4499

APPENDIX V

Plants per 3-yard length

Row No.									Total
1	5	4	5	3	6	6	7	6	42
2	7	4	5	5	4	7	5	6	43
3	1	6	4	4	5	5	6	6	37
4	5	4	2	3	3	5	6	4	32
5	4	1	3	4	1	2	4	5	24
6	4	4	7	5	5	7	6	7	45
7	6	3	3	4	2	2	2	2	24
8	7	3	4	6	2	1	2	5	30
9	5	4	2	3	2	3	1	4	24
10	5	4	3	4	3	2	7	5	33
11	5	5	4	6	5	6	3	4	38
12	7	3	2	5	2	3	4	5	31
13	3	7	4	2	3	7	4	5	35
14	6	5	2	2	0	5	2	6	28
15	6	4	5	2	2	4	6	6	35
16	5	6	5	4	4	4	4	5	37
17	3	4	5	3	6	3	5	5	34
18	5	4	1	6	2	2	2	3	25
19	4	6	4	6	7	4	4	5	40
20	1	5	3	4	5	5	0	7	30
21	6	5	6	1	3	5	3	4	33
22	3	6	6	6	4	4	2	3	34
23	8	6	7	5	3	6	3	6	44
									778

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TRIALS OF MERCURIC CHLORIDE FOR THE PREVENTION OF POTATO SICKNESS

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(With Plates V and VI)

The use of mercuric chloride as a soil dressing to prevent the attacks of certain insects and fungi is well known. Robson(3) found that heavy application of this salt to soil infested by *Heterodera marioni* successfully prevented infestation on tomatoes under glasshouse conditions.

The present writer observed that cysts of the potato strain of *H. schachtii* which had been immersed in weak solutions of mercuric chloride followed by washing in water lost their power of hatching in water containing potato-root excretions. For example, immersion of cysts in a 0·1 per cent. solution of mercuric chloride for 2 min. followed by washing in water for 3–5 min. had the effect of preventing hatching, whereas untreated cysts from the same soil hatched freely in water supplied with potato-root excretions. It was thought, therefore, of interest to investigate the effect of mercuric chloride applied to potato-sick soil. The following account summarises the results of this work carried out over a period of 4 years.

Experimental

Preliminary experiments were carried out in 1931 in a garden laid out for small-scale trials. The soil is a very light sand heavily infested by cysts of the eelworm. The garden was divided up into a number of plots separated by paths. Each plot was large enough to carry five potato plants and was bounded by boards, sunk to a depth of 9 in. in the soil, so that in effect each plot was a box full of infected soil. Nine plots were reserved for treatment, and three were available as controls. The mercuric chloride was used in the form of a solution, at three strengths, viz. 1 in 1000, 1 in 500, and 1 in 250.

The solutions were watered on at the rate of 1 gallon per square yard 3 weeks before planting. Immediately prior to planting, each plot received the equivalent of 8 cwt. per acre of a mixture of 2 parts of sulphate of ammonia, 4 parts of superphosphate, and 2 parts of sulphate of potash.

On the untreated plots the plants passed through the well-known stages of potato sickness and finally died away prematurely. The effect of the mercuric chloride as measured by the appearance of the plants was proportionate to the strength of the solution used. On the plots treated with the weakest solution (1 in 1000) the plants showed slightly healthier growth than those on the control plots. The 1 in 500 solution produced uniformly better plants, the symptoms of sickness being less marked and the period of growth being definitely longer. The effect of the strongest solution (1 in 250) was very striking, for the plants were the largest and most vigorous which had been grown at this centre since its institution in 1929. The yields shown in Table I supported the inferences drawn from observations.

Table I

Plot No.	Treatment	Yield in gm.
7	Mercuric chloride solution (1 in 250)	1562
8		1500
9		2053
4	Mercuric chloride solution (1 in 500)	443
5		612
6		476
1	Mercuric chloride solution (1 in 1000)	292
2		519
3		454
15	Nil	331
16		452
17		239

The table indicates that the treatment with the strongest solution caused a marked increase in yield as compared with that from the untreated plots. This increased yield is very considerable in view of the fact that the soil was almost entirely pure sand which had received no organic manure and had grown potatoes every year for 4 years.

It was observed at lifting time that the tubers from the treated plots were very clean and white, there being only the slightest signs of common scab and no sclerotia of *Corticium* (*Rhizoctonia*) *solani*. The untreated plots yielded tubers which were disfigured by scab and by large numbers of sclerotia of *Corticium*. Plate V, fig. 1, gives an idea of the size of the tubers obtained from a typical control plot and from a plot treated with the strongest dressing of mercuric chloride.

THE EFFECT OF MERCURIC CHLORIDE APPLIED IN THE SPRING

Trials were carried out near Selby in 1932 and repeated in 1933 to test the effect of an application of mercuric chloride a few weeks before planting. The site of the experiments was a field used for the large-scale experiments described by Buckhurst and Fryer (2). A single series of plots each 6 by 5 yards was laid down. The details of the treatments of the plots are given in Table II. In 1932 the chemical was applied in the powdered form 3 weeks before planting. In 1933 it was mixed with an equal weight of fine dry soil and applied 3 weeks before planting. In each case after even distribution the dressing was lightly raked into the surface.

At planting time the plots received a dressing of farmyard manure at the rate of about 15 tons per acre, and in 1932 the following mixture of fertilisers per acre: 2 cwt. sulphate of ammonia, 3 cwt. potash salts (30 per cent.), 4 cwt. superphosphate, $\frac{1}{2}$ cwt. steamed bone flour.

In 1933 the plots received the mixture of fertilisers used in the experiments described on p. 158.

Planting took place on April 15 in 1932 and on May 4 in 1933, and each plot carried two rows each of the three varieties Great Scot, Majestic and Eclipse. The crop was lifted when ready by hand.

EFFECT ON PLANT GROWTH

Observations made in the growing period during both seasons showed that the mercuric chloride had a serious depressing effect upon the early growth of the plants. Thus towards the end of June the treated plots presented a very disappointing appearance, the growth of the crop being uneven, many plants scarcely appearing above the ground and the foliage possessed a very dark green colour. This effect was less marked on the variety Great Scot. The untreated plots carried surprisingly well-developed plants. At this stage it seemed that this adverse effect due to mercuric chloride might be sufficient in itself to cause crop failure.

A month later the toxic influence of the chemical was still very evident and was more marked in the plots which had received the higher dressing. It was observed, however, that the variety Great Scot and to a less extent Majestic were beginning to grow out of the initial set-back. The variety Eclipse still lagged behind in growth, and in fact some plants had failed to grow at all.

During subsequent inspections the greater susceptibility of Eclipse to the effects of mercuric chloride in growth was all the more marked, for it was seen that the variety Great Scot and in 1932 the variety Majestic had made a comparatively good recovery and were making good growth in the tops. In the rows of Eclipse the uneven character of development persisted to the end of the growing season. That the phenomenon was in no way due to the "seed" was shown by the uniform germination and growth in the untreated plots.

By mid-June the control plots were showing the typical symptoms of potato sickness, and as the season advanced the plants gradually died off, leaving the treated plots still carrying vigorous plants. A striking result of the mercuric chloride treatments was the prevention of growth of weeds.

EFFECT OF MERCURIC CHLORIDE ON CYST INFESTATION

During both years specimen plants were lifted at intervals and the roots examined and an estimation of cyst infestation recorded. By mid-July all plants from untreated plots showed considerable numbers of cysts on the roots, whereas plants from treated plots were free from infection. During later inspections the control plots showed ever-increasing cyst numbers, but the degree of infestation always remained slight on treated plots. At lifting time the number of cysts produced on the control plots was very great, the tubers themselves being literally covered with cysts. Plants from the treated plots again showed only slight infestation of the roots, and the tubers were quite free from cysts. It seemed from these observations that the chemical treatment had largely prevented eelworm infestation. The absence of the summer stage of the fungus *Corticium* (*Rhizoctonia*) *solani* also was a feature on treated plots.

The results of cyst counts made on soil samples are given in Table II. Representative samples were obtained from the plots before planting and shortly after lifting by means of an auger, five borings from each plot being taken. The samples were dried and passed through a 2-mm. sieve, and the number of cysts in eight 20 gm. samples from each bulk sample was counted.

Table II

		Cysts in 20 gm. of dried soil			
		1932		1933	
Plot No.	Treatment	Before cropping	After cropping	Before cropping	After cropping
2	Nil	66·875	95·875	61·75	111·50
7		70·75	114·75	67·375	118·75
12		84·75	80·75	53·125	83·375
16		63·00	73·50	52·875	77·65
1	Mercuric chloride 0·64 oz. per sq. yd.	80·125	91·25	92·50	93·125
8		77·00	82·75	71·25	62·875
13		72·875	84·625	70·875	75·00
4	Mercuric chloride 0·80 oz. per sq. yd.	73·75	73·25	77·875	74·625
10		84·75	77·625	71·375	69·50
15		72·375	78·25	42·50	53·75

It should be remembered when considering these figures that in control plots large numbers of cysts were removed in lifting the tubers, the majority of which were heavily infested with cysts. It will be noticed that on all treated plots the cyst numbers show little variation over the whole experimental period. On the control plots, however, the soil sample taken immediately after lifting showed a very considerable increase in cyst content. The table indicates that this increase disappears during the winter, doubtless as a result of the disintegration of completely empty cysts. The stationary character of the cyst content on treated plots is quite compatible with the observed facts that there was only slight infestation of the plants on these plots, and therefore little complete emptying of the original cyst population, with the result that very few cysts would suffer disintegration.

Effect on yield

In 1932 the varieties Eclipse and Majestic were lifted on September 12 and Great Scot a week later. In 1933 the crop was lifted during the last week in August.

The yields are included in Table III.

Table III

		Yield in lb.					
		1932			1933		
Plot No.	Treatment	Eclipse	Majestic	Great Scot	Eclipse	Majestic	Great Scot
2	Nil	26	16	28	20	15½	22½
7		23½	14½	22	15	12	23½
12		26	17½	32½	21	17	28½
16		37	20½	32	18	9	26
1	Mercuric chloride 0·64 oz. per sq. yd.	34½	32	33½	24	20	29½
8		29½	27½	41	24	18½	31
13		20	25¼	37½	19	20	41
4	Mercuric chloride 0·80 oz. per sq. yd.	13	21	34½	19	12	28
10		20½	35½	36½	24½	16	43
15		24	30½	43	13	20½	37½

The yields support the inferences drawn from the observations recorded during the growing period, viz. that in spite of the toxic effect of mercuric chloride the variety Great Scot and to a lesser extent Majestic were stimulated by the dressing, and this was associated with a marked reduction in eelworm infestation and the accompanying signs of potato sickness. The variety Eclipse, on the other hand, although freed from eelworm infestation, lacked the power to recover from the effects of mercuric chloride and the yield suffered. It should be pointed out that the effect of mercuric chloride in general was to cause the production of large clean tubers, unaffected by common scab and the winter stage of *Corticium* (*Rhizoctonia*) *solani*. On all control plots the tubers were very considerably smaller.

THE EFFECT OF MERCURIC CHLORIDE APPLIED AT DIFFERENT TIMES BEFORE PLANTING

A new series of plots, of the same size as the 1932 series, was laid down alongside the 1932 series. Mercuric chloride was applied at three different periods of the year, the details being given in Table IV. In this case the mercuric chloride was mixed thoroughly with an equal weight of dry soil to facilitate even distribution, and was lightly raked into the surface.

The same three varieties were planted on May 4, 1933, when the plots received farmyard manure at the rate of about 15 tons per acre and the following mixture of fertilisers at the rate of 6 cwt. per acre: 4 parts of sulphate of ammonia, 3 parts of steamed bone flour, 3 parts of potash salts (30 per cent.).

Field inspections were made at about monthly intervals in both series of plots. Control plots and the plots treated with mercuric chloride in April showed precisely the same features as were recorded in the 1932 and 1933 trials previously described, viz. the control plots showed the usual symptoms of severe potato sickness accompanied by heavy root infestation by the eelworm, whereas the plants on the plots treated in spring were only slightly infested but suffered severely in the early stages of growth from the effect of the chemical.

On June 13, for example, the control plots carried plants 6–8 in. high, whereas the plants on the plots dressed in spring were only just peeping through the surface of the soil. Again it was noted that Great Scot showed a greater capacity for growing out of this set-back.

The effect upon growth, of dressing the soil in mid-December and the end of January was very striking. There was no set-back upon germination, so that on June 13 these plants presented a very pleasing appearance, the plants having germinated evenly and normally. After this date these plants quickly progressed and stood out the best in the whole area. The growth in the plots treated on December 13, 1932, was the same as the plots treated on January 31, 1933. These plants remained vigorous throughout the growing season, and the amount of tubers produced far surpassed anything that had been experienced on this field since it had been used as an experimental centre in 1929.

Examinations made for degree of eelworm infestation of the roots again showed heavy infestation on control plots both in roots and tubers, and only slight infestation in the plots dressed in spring. In the plots dressed in mid-December and late January the degree of infestation was decidedly higher than that of the spring-dressed plots but lower than that of the controls.

At lifting time it was noticed that the treatment of the soil in the December and January before planting had not prevented cyst infestation, apparently, to the same degree as the spring dressing, for cysts were found on the roots and sometimes on the tubers from the winter-dressed plots. On spring-dressed plots the comparatively slight infestation was again noticed. On the control plots the tubers were again liberally infested, and the root system was badly rotted and carried cysts in large numbers. The difference in the size of tubers from control and treated plots was again very pronounced. On control plots there was a preponderance of small tubers, on spring-dressed plots the tubers were fewer in number but of very good ware size, and on plots treated in December and January the tubers were more numerous and practically all of good size. Plate V, fig. 2 and Plate VI, figs. 1, 2, show produce of the three varieties from control plots and from plots treated with mercuric chloride at the three periods of the year. In the case of Great Scot and Majestic the very small tubers are placed in a pile in front of the heap of ware and seed.

The yields are given in Table IV.

Table IV

Plot No.	Treatment	Yield in lb.		
		Eclipse	Majestic	Great Scot
2	Nil	20	22	30
7		18	21	25
12		23	24	36
16		19	22	35½
3	Mercuric chloride	19	14½	37½
8	0·80 oz. per sq. yd.	18	17½	25½
13	April 1, 1933	15¾	21½	36½
5	Mercuric chloride	29½	30	44
10	0·80 oz. per sq. yd.	32	44	53
15	December 13, 1932	32	34	43
4	Mercuric chloride	29	38	40½
9	0·80 oz. per sq. yd.	34½	29½	47
14	January 31, 1933	35½	43	44½

The number of cysts in representative samples taken before and after cropping are given in Table V. The column of figures representing the number of cysts in the soil after cropping refer to samples taken immediately after cropping. Samples were not, unfortunately, taken in the following spring, so that the effect of the winter on the number of cysts cannot be determined.

Table V

Plot No.	Treatment	No. of cysts in 20 gm. soil: Before cropping	After cropping
2	Nil	50·00	81·25
7		79·25	149·75
12		61·125	70·625
16		62·25	104·50
5	Mercuric chloride	77·75	85·25
10	0·80 oz. per sq. yd.	80·375	97·375
15	December 13, 1932	66·75	79·25
4	Mercuric chloride	75·50	87·625
9	0·80 oz. per sq. yd.	89·00	100·875
14	January 31, 1933	79·875	75·50
3	Mercuric chloride	77·875	61·875
8	0·80 oz. per sq. yd.	78·75	111·625
13	April 1, 1933	71·625	64·875

The decided rise in the number of cysts on untreated plots as a result of cropping is again well marked, whereas the number of cysts in samples from treated plots suffers on the whole little change. Again, it should be pointed out that the tubers on the control plots carried large numbers of cysts which were removed at lifting time.

EFFECT OF MERCURIC CHLORIDE ON THE CYSTS IN THE SOIL

Observations in the field showed that treatment of the soil prior to planting decreased infestation by eelworm as judged by naked-eye examination of plants lifted at intervals during the growing season. This preventive effect was more marked the nearer the chemical was applied to time of planting.

An attempt was made to determine whether or not mercuric chloride had the power of killing the larvae enclosed in the eggs within the cysts.

Cysts were obtained during August 1932 from soil taken from plots 5, 10 and 15 immediately prior to treatment with mercuric chloride in December 1932. Similarly from the same plots cysts were obtained at

Table VI

Plot No.	Before treatment: Cysts used	Before treatment: Cysts hatched	After treatment: Cysts used	After treatment: Cysts hatched
5	45	27	45	15
10	37	25	37	26
15	29	21	29	24
Total	111	73	111	65

the same time from soil taken in April 1933, *i.e.* just before planting. The cysts were placed singly in glass capsules of water containing potato-root excretions, and the number of cysts from which living larvae were hatched were recorded.

The results are shown in Table VI.

It will be seen that mercuric chloride does not kill larval worms within the eggs. The beneficial effect of mercuric chloride cannot be ascribed, therefore, to its direct effect upon the cysts. It may act by deterring the larvae from hatching or by killing the larvae after hatching. In support of the suggestion that there was comparatively little actual hatching of the cysts in treated plots there is the fact that the cyst numbers remained stationary even immediately after cropping. On the control plots there was immediately after cropping a very definite increase in cyst numbers which disappeared during the ensuing winter as a result, it is suggested, of disintegration of empty cysts. If, as Buckhurst and Fryer suggest, there is in the production of potato sickness, in addition to the potato eelworm, another factor which is incapacitated by partial sterilisation, then the beneficial effect of mercuric chloride may be due to its capacity to neutralise this factor. The fact, however, remains that in the experiments described the prevention of potato sickness was accompanied by a marked reduction in eelworm infestation. It is also worthy of note that the reduction in eelworm infestation occurred in spite of a very severe check to early growth in the case of plots treated a few weeks before planting.

Buckhurst and Fryer suggest that a factor exists in potato-sick soils which by inhibiting vigorous early root growth renders the plants susceptible to serious eelworm attack. In the experiments described, spring applications of mercuric chloride most certainly inhibited early root growth, but they also prevented eelworm infestation, otherwise it seems safe to assume that the plants would have succumbed. Blenkinsop(1) has recently suggested that, in Cornwall and Devon, the important factor in potato-sick soils is not the eelworm but a high phosphoric acid-potash ratio. In view of this suggestion it may be of interest to quote the results of a small trial involving the application of both potassic fertiliser and mercuric chloride to potato-sick soil.

The trial was carried out in 1934 in a large garden near Leeds, the soil of which was heavily infested with potato eelworm and on which in 1933 the potato crop had failed. The history of the previous manuring showed that the garden had received inadequate supplies of potash. The experimental area consisted of eight small plots, each 8 by 2 yards, which received treatment as shown in Table VII. The sulphate of potash and mercuric chloride were applied on February 22, 1934 and Great Scot tubers were planted on April 10, 1934.

Table VII

Plot No.	Treatment	Yield in lb.
1	Nil	9¼
2	Sulphate of potash (1 oz. per sq. yd.)	18
3	Mercuric chloride (1 oz. per sq. yd.)	20¼
4	Mercuric chloride (1 oz. per sq. yd.) Sulphate of potash (1 oz. per sq. yd.)	35½
5	Nil	6
6	Sulphate of potash (1 oz. per sq. yd.)	14
7	Mercuric chloride (¾ oz. per sq. yd.)	18½
8	Mercuric chloride (¾ oz. per sq. yd.) Sulphate of potash (1 oz. per sq. yd.)	28½

As in other trials mercuric chloride delayed germination and early growth of the potatoes even when applied 6 weeks before planting. The plants recovered and subsequently made very good growth. Potash-treated and untreated plots produced poor, unhealthy plants with all the symptoms of potato sickness and which died off prematurely. Examination of the roots of specimen plants revealed only slight infestation on the plots treated with mercuric chloride. On all the other plots infestation on the plants was very severe.

The yields given in Table VII show that the application of potash alone increased the yield considerably, as was to be expected in view of the lack of potassic manuring of the soil in previous years. The produce from these plots, however, was like that from control plots, all of very small size. The tubers from mercuric chloride plots both with and without potash were small in numbers but of very good ware size, and unlike those from the remaining plots were free from common scab and *Corticium* (*Rhizoctonia*) *solani*.

The amount of potash applied is admittedly very small compared with the unusually heavy dressings applied in Blenkinsop's experiments. It is noteworthy, however, that the application of mercuric chloride produced a big yield of marketable tubers, and it seems hardly likely that this salt would affect the phosphoric acid-potash ratio of the soil.

It is perhaps worthy of emphasis that from the results of the trials described it is seen that when no dung is applied the effect of mercuric chloride dressing is most marked with regard to size and yield of tubers. The photographs of typical produce from the 1931 and 1933 experiments serve to illustrate this point.

SUMMARY

1. An investigation of the effect on potato sickness of mercuric chloride applied as a soil dressing is described.

2. It has been found that mercuric chloride applied 3–6 weeks prior to planting has a depressing effect on the early growth of potatoes. The variety Great Scot withstands this effect better than the varieties Majestic and Eclipse. This toxic effect is prevented by applying the chemical 3 or 4 months before planting.

3. The degree of infestation of the plants by the eelworm is decreased by dressing the soil with mercuric chloride. The reduction in infestation is greater the nearer the application is to the time of planting.

4. The reduction in eelworm infestation is accompanied by an increased yield of tubers except where plants cannot properly recover from the direct toxic effect due to mercuric chloride.

5. Evidence has been obtained to show that mercuric chloride does not kill the eggs within the cysts.

6. Mercuric chloride treatment of soil effectively prevents the fungous diseases common scab and collar rot.

The writer wishes to acknowledge with thanks the assistance given by many of his colleagues, and especially to Mr W. E. Gelling and Mr J. Strachan for their help in arranging the field trials, and to Mr S. Burr and Dr W. A. Millard for their help with the mycological aspect of the investigation.

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EXPLANATION OF PLATES V AND VI

PLATE V

Fig. 1. Total crop from: *a*, plot 15, untreated; *b*, plot 7, mercuric chloride. (See Table I.)

Fig. 2. Variety Eclipse. Total crop from: *a*, plot 16, untreated; *b*, plot 15, mercuric chloride applied December 13, 1932; *c*, plot 14, mercuric chloride applied January 31, 1933; *d*, plot 13, mercuric chloride applied April 1, 1933. (See Table IV.)

PLATE VI

Fig. 1. Variety Majestic. Total crop from: *a*, plot 16, untreated; *b*, plot 13, mercuric chloride applied April 1, 1933; *c*, plot 14, mercuric chloride applied January 31, 1933; *d*, plot 15, mercuric chloride applied December 13, 1933. (See Table IV.)

Fig. 2. Variety Great Scot. Total crop from: *a*, plot 13, mercuric chloride applied April 1, 1933; *b*, plot 14, mercuric chloride applied January 31, 1933; *c*, plot 15, mercuric chloride applied December 13, 1932; *d*, plot 16, untreated. (See Table IV.)

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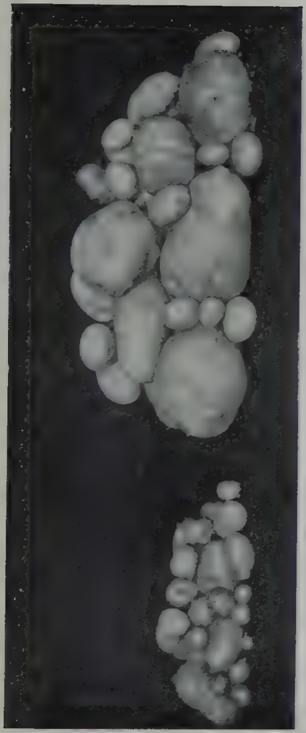

Fig. 1.

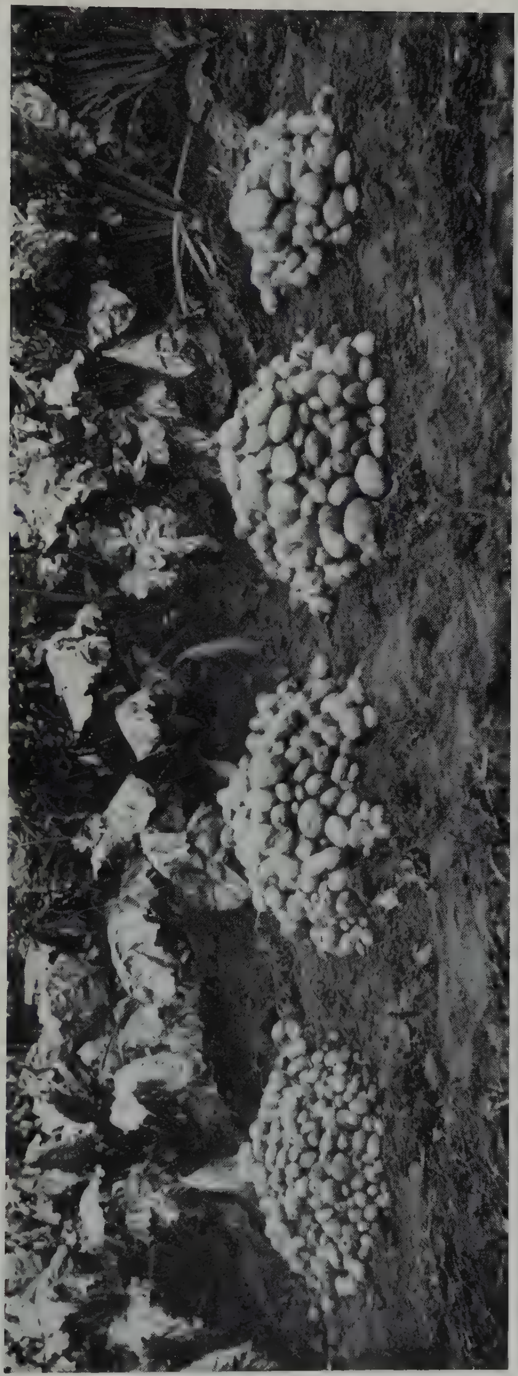

Fig. 2.

JOHNSON.—Trials of Mercuric Chloride for the Prevention of Potato Sickness (pp. 153–164).

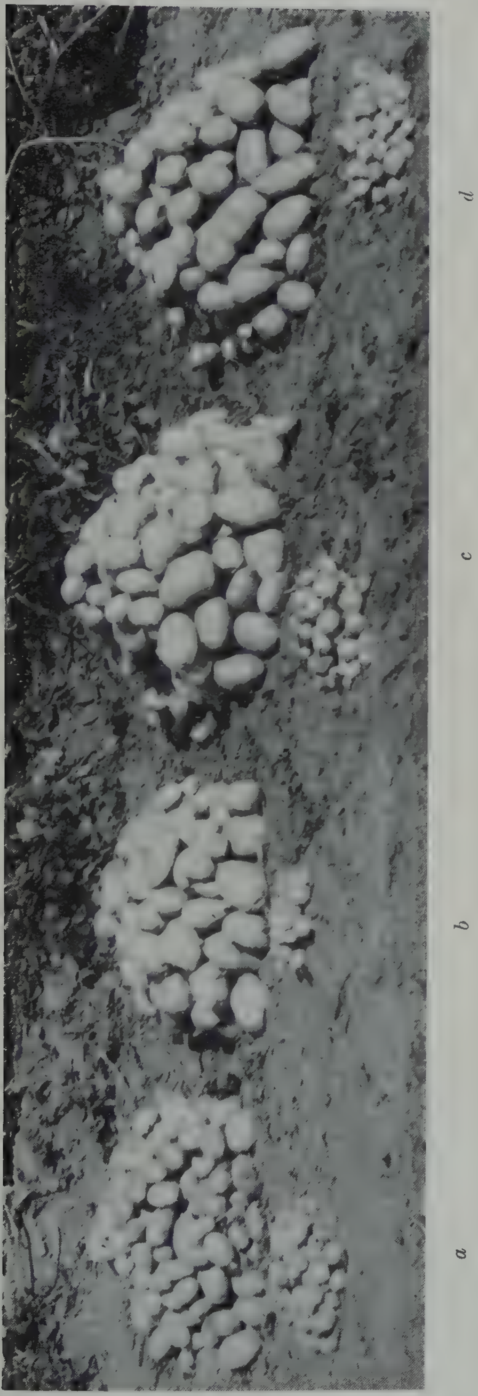

Fig. 1.

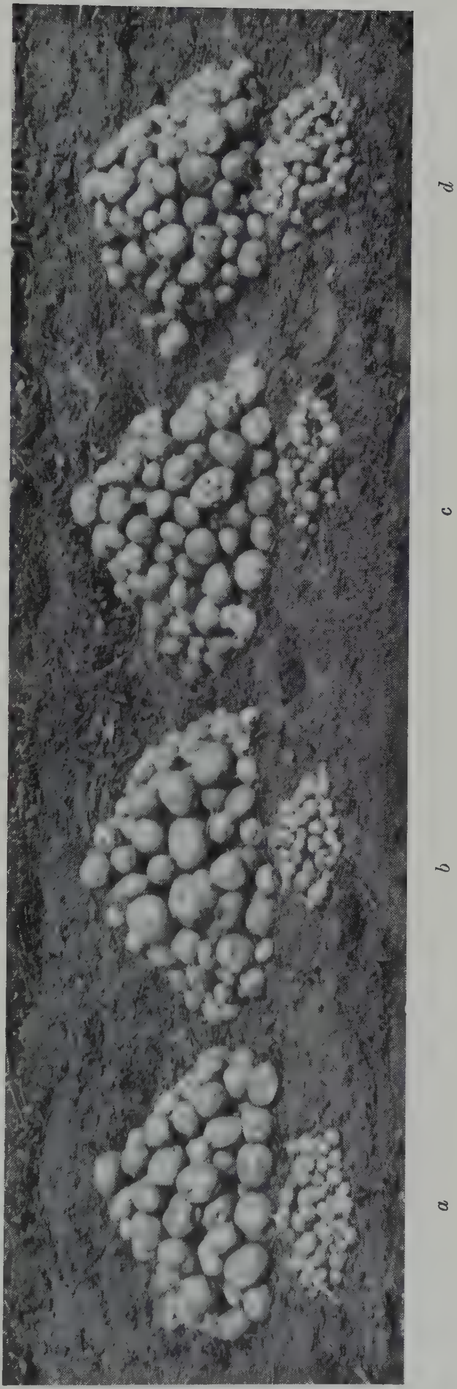

Fig. 2.

JOHNSON.—Trials of Mercuric Chloride for the Prevention of Potato Sickness (pp. 153–164).

METHODS OF DETECTING MUSKRATS

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(With 2 Text-figures)

I. Introduction

The successful trapping of muskrats (*Ondatra zibethica* L.) (whose spread and present distribution in the British Isles have been described in a previous paper, 1934) depends to a great extent upon the ability of the trapper to recognise the signs of their presence as an aid to placing his traps.

Although any well-trained trapper is already aware of many signs which can be recognised, it was thought to be of advantage to summarise some of the known methods of detection, together with some other methods which have been evolved in the course of research surveys in Shropshire and Scotland, carried out for the Bureau of Animal Population under a grant from the Ministry of Agriculture and Fisheries.

All the three muskrat campaigns in Britain began work on a dense population of animals. This was in each case greatly reduced, till, by the summer of 1934, muskrats were very scarce in Perthshire and Shropshire and almost exterminated in West Sussex and Surrey. Signs of muskrats which had been obvious to field workers at the outset have become scarcer and the animals extraordinarily elusive.

II. Methods of detection

Houses. A house is irrefutable evidence of muskrats. Owing to tall marsh vegetation it is sometimes not as easily found as might be expected. In Britain, houses may be begun as early as the end of September and, later, during October and November. They are made of materials such as mud, matted roots, and stems of reeds, and even dead leaves of trees.

"*Push-ups*". In Scotland other signs less obvious than houses, but just as good evidence of the presence of muskrats, may occur in the marshes and back-waters. These signs are known to the trappers as "push-ups", and are made in the winter. They seem to be almost invariably connected with the course or entrance of a channel running in the water or liquid humus underneath the surface mat of vegetation. They consist of mouthfuls of matted roots placed round a small hole. An apparently more finished type shows a dome over the entrance, rising to a height of 3 or 4 in., and is also made of dead vegetation. Small heaps of surface humus may also be scraped together, not necessarily in connection with burrows or searchings after roots.

Plugs. Muskrats often pull up water plants for plugging up the entrances of burrows not in use, stopping gaps caused by cattle or man, and covering parts of runways amongst tangled tree roots. This is a habit not shared by any other British mammal.

Food-traces. With signs such as bitten-off vegetation we find ourselves on less sure ground. Muskrats inhabiting a pond in small numbers may leave either ample sign of their presence or next to none, depending apparently partly on the time of year, and partly on the duration of the infestation. A single muskrat entering a pond in the migratory season may leave more sign of its presence than a small resident colony would do. In autumn a general collecting of food materials takes place, and much that is cut floats to the surface of the water. The muskrat eats almost any aquatic or succulent bank vegetation, but roots are nearly always preferred to the upper parts of the stems and leaves. This accounts for the large quantity of floating material sometimes found, consisting mostly of the thinner stems and leaves. It is almost impossible to tell from an examination of the smaller species of plants in this floating mass whether muskrats or aquatic wildfowl are responsible. It is often possible to say from the cut ends of the larger species of rushes, reeds, and grasses, whether or no a rodent has been at work; but with the exception noted below it is impossible to be at all definite. The roots and stems of water lilies (*Castalia alba*) are favourite foods. The stems are often pulled up by swans, etc., but are of too pulpy a consistency to give any sign characteristic of muskrat incisors. The presence of its fleshy rootstocks floating on the surface is suspicious, as they are too stout to be easily dislodged from the bottom of the pond. White shoots of the underwater parts of the great reed mace (*Typha latifolia*) are often cut off by the muskrat and float to the surface. The thick stems of the bulrush (*Scirpus lacustris*) are cut off in a characteristic manner, the cut surface being at an acute angle to the length of the stem, and presenting a series of definite steps, each of about 2 mm. height. The stem may be cut cleanly through or may have a slice of cortex left upstanding. Water voles (*Arvicola amphibius*), in gnawing this plant, find the stem too thick for a frontal attack, and bite first on one side, then on another. The bites are smaller and the general effect is untidier. The trailing ends of long pieces of plant fragments not completely dragged inside the burrow may sometimes be seen. Unless there is a small colony, it is unlikely that much cut-off floating material will be found caught by boughs dipping into the water. On rivers swans, ducks and other birds and stock dislodge a great deal of aquatic vegetation, which is constantly floating downstream, and which is hardly distinguishable from that cut off by muskrats. These, however, often cut much greater lengths of stem than do birds, and there are as a rule no roots attached. Sometimes in rivers with thick beds of aquatic vegetation near the surface the actual places where muskrats have removed plants can be found.

Muskrats along river (and pond) banks bite off shoots of willow (*Salix*) in a similar manner to that already described for bulrush. In the case of twigs ¼ in. or more in diameter the method of cutting is apparently characteristic of the animal. Shoots springing from boles near the water level (especially in the neighbourhood of burrows in use) are the most often attacked. This is a most useful sign of the presence of muskrats.

Burrows. The burrows of the muskrat, except those made by the young animals, are appreciably larger than those of the water vole and brown rat. The width of the runway used by an adult muskrat is usually 4–4½ in. A new burrow, at its narrowest point, may be no wider than this. The size of the same burrow varies considerably and increases as time goes on. This increase depends on the extent to which it is used and to the degree of water erosion. Burrows are usually higher than wide, Stock often break through the roofs, especially around ponds, and this may be the first sign of the presence of the animals. The air hole is not invariably present and when plugged with weeds is hard to see. The burrows in use seldom open above water except when the entrance is well concealed by overhanging tree roots. If an entrance in use is laid bare by a fall in the level of the water, it is often plugged up with vegetation, and another one made underneath the new level. Entrances to burrows may lie from just under the water level to a depth of 3–4 ft. They may open not on the surface of the bank, but in the bed of the pond or river, at a distance of as much as 10 ft. from the bank. A tuft or bed of aquatic vegetation, through which the muskrat passes as through a curtain, may hide its entrance. The entrances to burrows in ponds where the bottom is soft may be betrayed by valleys in the mud leading to them, caused by the constant passage of the animals. (In rivers of rather quiet flow and muddy bed, the mud in front of the entrance to a burrow which is in use is softer than that in the immediate neighbourhood, owing to frequent disturbance.) These valleys may contain turbid water, even when the rest of the pond is clear. Paths through the surface weeds or floating debris, and bubbles under the ice when the pond is frozen also help to locate the entrance to the burrow. The heaps of excavated earth which one might expect to find on the bed outside the entrance are often surprisingly little in evidence. The finding of burrows and general detection of muskrats along the banks of rivers with moderate current is for rather obvious reasons much more difficult than on ponds.

Artificial aids in detection. For systematic examination of pond and river banks for muskrat burrows, where it is difficult to search any other way, and where obvious signs are few or lacking, Roith's "Fangerstöck" or probing bar is employed. This is essentially a straight iron rod with a crook at one end to act as a handle, and a tapered bulbous tip at the other. When the rod is thrust into solid ground uniform resistance is met with; but when a burrow, crack, etc. is encountered no resistance is felt and the rod slides down easily. The size, plan and depth under the soil surface of the burrow can be found. In suitable soils, some trappers claim, by an examination of the upper part of the bulb of the probe, to be able to form an idea as to whether the burrow is in use or not. If the burrow is unused and silted up, this part of the rod is covered with liquid mud. In the present state of affairs when very few muskrats are abroad, the probing bar is very useful in the detection of new burrows. Old burrows are as a rule kept trapped in case they are visited by an odd wandering animal. The method has its limitations. It is a practical impossibility to probe systematically everywhere, while hard and stony soil and tree roots add to the difficulties. The depth at which burrows may be detected is of course limited by the length of the bar. In Scotland a water telescope similar to those used by the collectors of pearl mussels has been used successfully, but on a rather limited scale, to locate burrows.

Footprints. A clear impression of the footprints of a muskrat is unmistakable. The large hind-foot may show the impression of the web; and in the case of an animal walking over soft mud the under edge of the tail leaves a line. The few footprints now made are however often obliterated by those of the commoner waterside animals such as water voles, brown rats, and waterhens. Footprints are not necessarily found, even where a small colony of muskrats is present; in fact, when muskrats are scarce they rarely seem to leave the water. The young are for a few weeks much more terrestrial in habits and their footprints are therefore relatively more often found. The bed of a shallow quiet part of a river, where muskrats are swimming in a definite route, will sometimes show a trail of mud lighter coloured than that of the surroundings, even though no turbid water is present.

Bubbles under ice. The bubbles made by the muskrat while swimming under ice vary in size from about ½ to 1½ in. in diameter. They are not released continuously, but only now and then, especially when the muskrat comes to the bank. When only one or two muskrats are living in a pond observation of bubbles under the ice is of great help, both in detection of burrows in use and trapping. The parts of the pond most frequented by the muskrat can be found and traps laid accordingly.

III. Examination of dung

The importance of finding a method for the accurate identification of muskrat dung was realised early in the course of the present investigation. Fortunately, in Britain there are only two other waterside animals whose excreta could be confused with those of the muskrat: these are the water vole (*Arvicola amphibius* L.) and the brown rat (*Rattus norvegicus* L.). These three animals differ greatly in size one from the other. As they all subsist more or less on a vegetable diet, it might be supposed that the size of particles of plant fibre in the excreta would increase from water vole to brown rat, and brown rat to muskrat. This is generally true, but at the same time can be no more than a guide. The particle size of the excreta depends on the character of the food, the size and perhaps the type of molar teeth which the animal possesses. In young muskrats just abroad for the first time the particle size is much the same as that in the adult water vole.

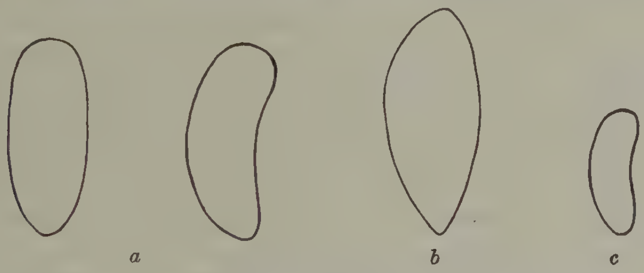


Fig. 1. *a*, muskrat dung, × 1⅓. *b*, brown rat dung, × 1⅓. *c*, water vole dung, × 1⅓.

Appearance of fresh dung. Typical faecal pellets of muskrat, water vole and brown rat are shown in Fig. 1. Brown rat dung is usually lighter and browner than that of the other two species and has when fresh an unpleasant smell. Both water vole and muskrat have a smaller variety of dung which is stouter and straighter than that figured. The dung of adult muskrats varies from 14–23 mm. in length and is about 7 mm. in diameter. The excreta of young muskrats which have just begun to fend for themselves are indistinguishable externally from that of adult water voles.

Habits of defaecation. The habits of defaecation greatly affect the extent to which the dung identification method can be employed in practice. The faeces of brown rats are not so often found at the waterside as are those of water voles. This is more likely to be due to a habit of defaecating when swimming than to relative scarcity. Pellets, mostly single ones, are to be found in runways or on horizontal boles of willow trees above the water. Water vole dung may also be found in such situations; but where the animals are at all abundant plenty of it is to be seen. The water vole has regular dunging places where recent dung may be found laid on a trampled mass of older excreta. Such places may occur anywhere on a run; but especially near (or even on) a feeding place, and just above a vertical run into the water. Defaecation also takes place in the water. Muskrats too have favourite places for defaecation, where a cake of excreta may be found and identified by its contained hairs. Such places are usually tree trunks just above the water. Isolated pellets may also be found on horizontal tree boles, or the low crowns of willows. There is no doubt, however, that most evacuations take place while swimming. Consequently, in a deep river like the Severn, dung traces are scarce. When marshes, shallower streams or ditches are infested pellets are more often found. A marked difference with regard to defaecation occurs in the young. Young muskrats feed on the grasses and other vegetation of the river bank to a much greater extent than the adults normally do, and they defaecate in runways by the waterside, much as do water voles. Also like the latter animals the young muskrat may evacuate a little group of pellets at once. When the stomach contents of muskrats were being investigated it was noticed that fine hairs were invariably present. Hairs were also found in the stomach contents of water voles and brown rats. The excreta of these three species also contain hairs. A method of identification of dung is given below, based on the microscopic differences between hairs in the three species.

Macroscopic examination. Dung collected in the field is placed in a small box and allowed to dry. Hairs present on the outside of the pellet are first removed. The pellet is moistened with water—just sufficient to enable it to be pulled apart easily with small forceps, the pellet being held between the fingers or with a pair of larger forceps. A little is removed at a time and when a hair is found it is removed and placed in a watchglass containing a little 70 per cent. alcohol. Hairs may be examined therein, or mounted in Canada balsam in the usual way. It may be said that, with the exception noted below, a single pellet of dung yields a sufficient number of hairs for identification purposes. Several pellets are usually found together in the field.

The hairs from brown rat faeces are noticeably coarser than those from water vole and muskrat. Some of the body hairs are distinctly whitish, or white with a narrow dark zone in the middle. The wool hairs have markedly angulated waves. In the water vole the wool hairs, which are by far the commonest type found, are usually wavy and fine in texture. Faeces of the muskrat yield very many wool hairs, which are usually more wavy and often longer than those of the water vole. Shorter wool hairs may be found, but most pellets contain a sufficient number of very long wool hairs which put the issue beyond doubt.

Microscopic examination. Measurements of two series of sixty wool hairs from each of the three species were taken. One series was removed from skins, and to be as representative as possible ten hairs from each of six skins were used in each of the three species. The other series was removed from dung; in the case of the muskrat, this was obtained from the recta of several individuals, while the water vole and brown rat excreta were collected in the field. The ratio, width of medulla to width of hair was found. Table I and Fig. 2 show the results obtained. The following points emerge:

(1) Muskrat hairs are less variable as regards the ratio measured than are those of water vole and brown rat.

(2) Owing to the degeneration of the medulla in the hairs obtained from faeces these hairs show more frequently a lower medullary width ratio than do those from skins.

(3) Muskrat hairs are well represented (as compared with the other two species) in the 0·30–0·50 ratio class, about equally in the 0·50–0·70 class and not at all in the 0·70–0·90 class.

The ratio medullary width to hair width for the range 0·30–0·50 therefore affords a reliable method for distinguishing the wool hairs (and hence the faeces) of muskrats from those of water vole and brown rat. The number of muskrat hairs which fall into this ratio range is about double that of either of the other two animals. The above method is applicable

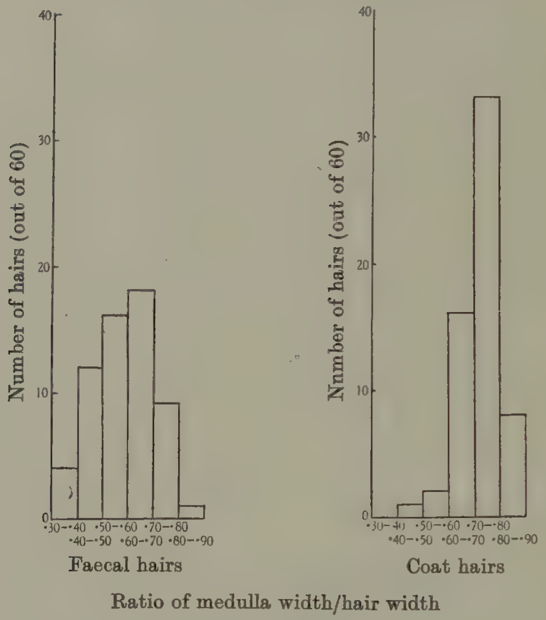


Fig. 2 *a*. Water vole

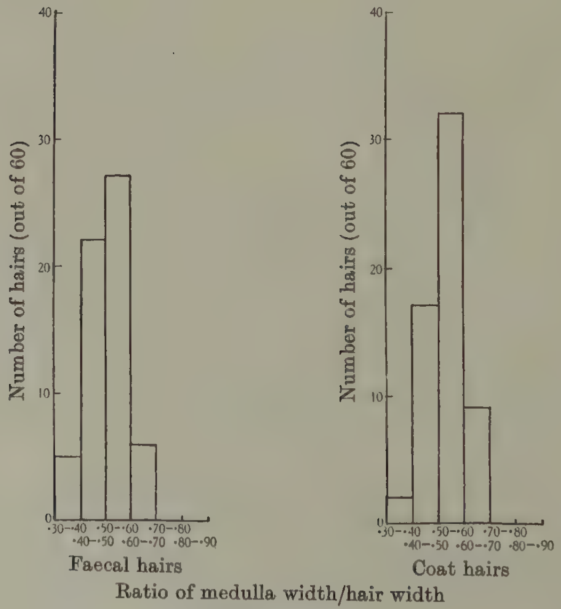
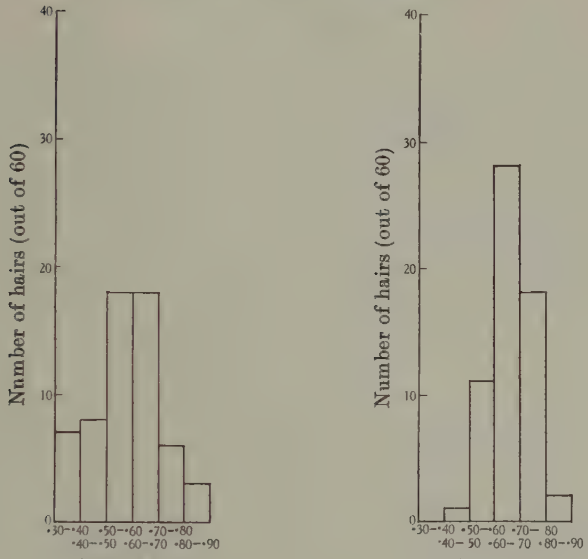


Fig. 2 *b*. Muskrat.



Faecal hairs Coat hairs

Ratio of medulla width/hair width

Fig. 2 *c*. Brown rat.

Table I

Ratio of medulla width/hair width. Number of hairs out of 60 *falling into the different ratio groups*

		0·10–0·20	0·20–0·30	0·30–0·40	0·40–0·50	0·50–0·60	0·60–0·70	0·70–0·80	0·80–0·90	0·90–0·100
Muskrat	Coat hairs	—	—	2	17	32	9	—	—	—
	Faecal hairs	—	—	5	22	27	6	—	—	—
Water vole	Coat hairs	—	—	—	1	2	16	33	8	—
	Faecal hairs	—	—	4	12	16	18	9	1	—
Brown rat	Coat hairs	—	—	1	11	28	18	2	—	—
	Faecal hairs	—	—	7	8	18	18	6	3	—

to old faeces which have lost their natural shape. In one case (that of young muskrats) the method largely breaks down. Examinations of the dung of young muskrats for hairs have been consistently negative. Very few hairs have been found and these were short and insignificant. Fortunately young muskrats often leave at the same time, as well as dung, other signs of their presence, such as footmarks. The dung of young water voles is also hairless and is noticeably smaller than either that of adult voles or young muskrats.

IV. Summary

In this article natural signs left by muskrats and various artificial aids in detection are described and discussed. Houses and the so-called "push-ups" are in marshes obvious and characteristic signs. With few exceptions it is hard to state definitely whether food traces found in the field have been made by muskrat or not. Willow shoots and the stems of bulrush (*Scirpus*) are however bitten off in a characteristic manner. The finding of burrows is very important from the practical standpoint. Where the burrows are not superficial the Fangerstöck or probing bar is employed in locating them. The footprints of the muskrat are unmistakable.

A method for the identification of muskrat dung is given. This depends on the measurement of the ratio of medulla width to hair width in hairs obtained from the faeces and its comparison with that of similar hairs from water vole and brown rat. Far more hairs of the muskrat lie in the ratio limits 0·30–0·50 than do those of water vole and brown rat—the reverse being true of the ratio limits 0·70–0·90.

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PROCEEDINGS OF THE ASSOCIATION OF APPLIED BIOLOGISTS

General Meeting of the Association of Applied Biologists held in the Botany Lecture Theatre of the Imperial College of Science and Technology, South Kensington, London, on Friday, October 11, at 11.45 a.m. The President, Dr T. Goodey, in the Chair.

The following papers were read on "Some Glasshouse Problems at the Cheshunt Experimental Station".

I. Introduction. By W. F. Bewley, C.B.E., D.Sc.
II. Animal Pests of Interest in Glasshouses. By E. R. Speyer, M.A., F.R.E.S.
III. Some Observations on the Composition of Tomato Plants. By O. Owen, M.Sc., Ph.D., A.I.C.
IV. The Control of Red Spider Mite and Tomato-leaf Mould. By W. H. Read, M.Sc., A.I.C.
V. Some Important Diseases of Glasshouse Plants. By P. H. Williams, B.Sc.

I. INTRODUCTION

By W. F. Bewley, C.B.E., D.Sc.

The Experimental and Research Station, Cheshunt, was conceived during that period of optimism which occurred prior to the war; it was born when the war clouds were gathering over Europe, and saw its early childhood during the time of international strife.

Essentially the child of the Lea Valley Growers, it soon developed ambitions beyond its home circle and is now recognised as the National Station for crops grown in glasshouses and in frames. Its origin and development have been governed by the needs of the great industry it is called upon to serve.

The Lea Valley area was commenced in 1876, when the late Mr Joseph Rochford went to Turnford and built the first commercial glasshouses in that district. During the next thirty years, when the industry was developing, there appears to have been no great demand for scientific research, owing no doubt to the fact that the crops then grown were raised under more natural conditions than they are to-day.

Later, economic changes led to more severe forcing and the production of "out of season" crops. During the early part of the present century, problems of pests and diseases in addition to that of "soil sickness" confronted the growers, and it was natural that they should turn to science for the help which they needed so badly.

They found a very sympathetic friend in Sir John Russell, who was instrumental in introducing to them the process of soil sterilisation—a process which proved most successful and which has developed out of all recognition during recent times.

Having found a means of overcoming one of their troubles, the Lea Valley Growers were not slow to realise that organised research was vital to the security of their industry, and in 1914 the Cheshunt Research Station came into being.

It is not my intention as the opener of to-day's proceedings to do more than introduce you to the station, point out the nature of its work, and lightly touch upon some of the more important results which have been obtained in the past.

The success of the station is largely dependent upon the close co-operation which exists between the scientist and the grower, and it is natural that the greater part of the work must be of a very practical nature. At the same time the more fundamental side of the work is not forgotten, for upon it must depend any future improvements in heating, construction and crop management. Thus while each member of the staff must take a share in the advisory work, he or she is responsible for a definite section of the more fundamental investigations.

The period 1914–19 was one of great difficulty because, owing to the national crisis, it was difficult to find either the men or the money for the work. When the year 1919 dawned, hostilities had ceased and the nation settled down to a process of reconstruction and development. Diseases and pests had developed and spread considerably during the war years, and it was the ravages of the tomato moth caterpillar which led to the appointment of the first entomologist, Dr L. Lloyd, in 1919.

This pest, on the growers' own estimate, was costing the Lea Valley Growers the sum of £40,000 per annum by the destruction of plants and fruit and the cost of hand picking the caterpillars, the only means of control then adopted.

Dr Lloyd quickly devised control measures involving the trapping and destruction of larvae during the winter, the trapping of moths as soon as they emerged, and the spraying of plants with arsenate of lead during the early stages of growth. The measures he recommended were adopted whole-heartedly by the growers and proved most successful. The destruction of plants and fruit was reduced to negligible proportions by the annual expenditure of a few hundred pounds only.

Another pest of glasshouses, the greenhouse white fly, was causing damage valued at £25,000 per annum in the Lea Valley. Little attempt was made to control it, because although many growers had tested the method of "cyaniding" recommended for this pest, the plants had been injured so badly that they refused to try it again. Dr Lloyd perfected a method of "cyaniding" tomato houses so as to obtain a maximum kill of the insect, at the same time reducing to a minimum any risk of injury to the plant from the poisonous gas generated. As an alternative to "cyaniding", especially in cases of less frequent attacks by white fly upon cucumber plants, which will not stand fumigation at full dosage, Dr Lloyd introduced the liquid fumigant Tetrachlorethane.

In 1926, a few scales of the greenhouse white fly parasitised by a minute chalcid wasp *Encarsia formosa* Gahan, were sent to the station by Mr L. Hawkins through the kindness of the *Gardeners' Chronicle*. Mr E. R. Speyer investigated the life history of this parasite and devised means whereby it could be bred on a large scale for distribution to growers. Its success was immediate, and the work of breeding and distribution, now part of the routine of the station, is in the hands of Mr O. Orchard.

The parasite has been distributed from the station to all parts of the British Isles, and to distant colonies.

The work on manurial treatment and crop management has yielded many results of value to the industry, but probably the most important are those which showed the necessity for regulating the application of fertilisers in accordance with the weather conditions.

It was demonstrated that the total weight of the tomato crop in any year is dependent upon the total hours of bright sunshine between April 1 and September 30, and that during hot sunny weather the tomato requires a good deal of nitrogen and a little potash, whereas in dull weather it needs heavy applications of potash and but little nitrogen.

The last result, which suggests that lack of strong sunshine can be compensated to some extent by applications of potash, has explained the apparent contradictions between the results of manurial trials in the hot sunny areas of America and those in the less favoured parts of the world. In the former, heavy applications of nitrogenous fertilisers are essential, while in the duller areas of Europe heavy dressings of sulphate of potash are most necessary. It also shows that the tomato in this country requires a good deal of potash and little nitrogen during the early stages of growth, with increasing amounts of nitrogen and less potash as the plant ages.

These results have been of the greatest assistance in advising successful treatments for different parts of the country and for different types of seasons. The work on tomatoes is being extended to other crops and shows without doubt that manurial treatment and crop management must be regulated as the weather conditions demand.

Investigations into the effect of raising the temperature of glasshouse soils began in 1927, when Mr Olsen of the Scandinavian Cable Works at Oslo presented a set of cables similar to those used by him for supplying bottom heat to frames. Preliminary experiments quickly showed that the temperature of glasshouse soils in this country is lower than is desirable, and that considerable improvement in plant growth and crop production can be obtained by increasing it artificially. Root development is greater, the roots are cleaner and live longer than in soil at ordinary temperatures. Vegetative growth is more vigorous, and the plants remain green and healthy beyond the time at which they are usually finished. The first box of tomatoes is not usually picked sooner, but the main crop ripens earlier and a greater weight is picked during the first month. In the case of cucumbers heavier and earlier crops have been obtained.

A high percentage of the root diseases which have made soil sterilisation so necessary are the result of unfavourable physical and chemical conditions, which appear to be corrected by raising the soil temperature. On the other hand, some diseases would not be affected and certain pests such as the eelworm and wireworm might become more serious. It would seem that soil warming will ultimately replace soil sterilisation to some extent in this country, where low temperature conditions prevail.

The electric method employed in the preliminary experiments is too expensive at the present cost of electricity for it to be applied to commercial tomato and cucumber work, but a suitable method has been devised for warming the soil by circulating hot water in small pipes buried beneath the soil. Experiments on a commercial scale have been conducted in various parts of the country, and it seems likely that the method will ultimately become standard practice.

The hot water is prepared in a separate boiler from which it is led by 2-in. mains to a series of pipes 1 in. in diameter placed 3 ft. apart and buried 2 ft. below the surface of the soil. Circulation is effected by a small electric pump fitted with a stopping and starting device controlled by a thermostat placed in the soil. By this means the soil temperature can be maintained constantly at the desired temperature.

Another important part of the work of the station was the raising of a new autumn and winter lettuce, Cheshunt Early Giant. Prior to this there was no variety which, when grown to be harvested between December and February, would form a sufficiently large and solid heart to satisfy the requirements of the market; and the public was dependent upon imported lettuce for its supply. This new lettuce can be cultivated at a time when the glasshouses of this country are usually empty. It can be grown to produce 6- or 7-oz. lettuce with a large solid heart for Christmas and the next 10 or 12 weeks. It has met a popular demand and provided the home grower with a means of competing with importations from abroad.

Time will not permit a fuller discussion of the work at Cheshunt, but it must be borne in mind that as the station is intended to serve the glasshouse industry to the fullest possible extent, its activities must cover a wide field. In addition to problems directly concerning the crops themselves, problems of house construction and building materials, heating and fuel values have been investigated as the need arose.

Supporters of the station are drawn not only from the Lea Valley, but from every corner of the British Isles, and from various parts of the Empire.

II. ANIMAL PESTS OF INTEREST IN GLASSHOUSES

By E. R. Speyer, M.A., F.R.E.S.

It is intended here to refer briefly to one or two animal pests of glasshouse plants, of which little notice is taken nowadays, owing to control measures devised for them having been employed with some measure of success by the glasshouse industry.

The problems of the present day, however, cannot be disregarded, and that particularly concerned with injury to flowers of ornamental plants by Thysanoptera, commonly known as "Thrips", might offer material for useful discussion.

Lastly, some reference to the biological control of pests in glasshouses and its possibilities should not be out of place.

Woodlice

Not perhaps of particular interest biologically, these Crustacea were in 1922 so prevalent in cucumber houses in the Lea Valley, that measures for their control had to be undertaken. The injury caused by them was at first not very apparent, but it came to light that the period of cropping was seriously curtailed through the removal of the outer layers of the stem. Though present in incalculable numbers, the species particularly responsible for the damage was found by Dr Jackson to be new to science, and was described by him under the name *Armadillidium speyeri*.

An inquiry into the feeding habits of this and other species showed that they were not particularly attracted to any form of bait commonly in use, but that they would readily feed upon dry bran, a cheap commodity which is easily distributed so as to cover the soil in which the woodlice live.

Of the poisons tested with a view to mixing with bran, Paris green and potassium bichromate were effective, but the latter was so distasteful to the animals that baits made up with it were never entirely efficient. Paris green had long before been used in baits for woodlice, but was also found to be repellent if contained in too great quantity in the bait. The best proportions were determined as Paris green 1 part to bran 28 parts, a mixture which killed the woodlice in thousands when broadcast over the borders and paths at the small rate of about ½ oz. per square yard. Several years later it was found that dried blood, a most valuable top-dressing manure, was often more attractive than bran, and the best proportions here proved to be Paris green 1 part and dried blood 56 parts. Paris green baits are effective against other kinds of woodlice, such as *Porcellis laevis* Latr., often brought into glasshouses in stable manure, *Armadillidium vulgare* Latr., usually common in tomato houses, and *Oniscus asellus* L., which is injurious to orchids.

When new glasshouses are erected upon grassland, it sometimes happens that the common pill-woodlouse, *Armadillidium vulgare*, is present in the soil in vast numbers, and may then constitute a serious pest.

Such an occurrence was recorded in May 1925, when cucumber plants upon a new nursery in the Lea Valley were almost entirely stripped of their foliage. By means of empty seed boxes placed upon the borders, the grower concerned had collected some 4 cwt. of the animals up to June 11, this representing about 2½ million woodlice, but no material decrease in their numbers was apparent. On this date the borders were treated with the Paris green bran bait, and after a second application the grower reported 6 days later that the woodlice had to all intents and purposes vanished.

Chrysanthemum midge

Diarthronomyia hypogaea Low. is a cecidomyid fly which has caused considerable loss to growers of chrysanthemums in the United States. The larvae form characteristic galls upon the foliage, and often upon the stem to an extent which leads to deformation of the flower shoots. The insect was probably not recorded in Great Britain prior to 1927, but in October of that year an outbreak occurred in Hertfordshire and Essex, and this was traced to the importation of cuttings of the variety Monument from the United States three years previously. From the subsequent distribution of cuttings amongst growers, eight nurseries became infected, and the insect spread to some ten varieties, three of which were subject to severe infestation.

Co-operation between the Ministry of Agriculture, the research station and the growers concerned led to the immediate adoption of control measures recommended by the Cheshunt Station. These were:

(*a*) Spraying twice weekly with commercial nicotine (90–98 per cent.) 1 part in 400 parts water, with potash soft soap ½ oz. to the gallon, up to the time of the opening of flower-buds. This killed the eggs (which are laid near the growing point of young shoots) and the adult flies emerging from the galls, but left many larvae within the galls alive.

(*b*) Removal and burning of all young growth from the base of the plant toward the end of December. It is now considered that this was the most important step which led to eradication of the pest.

(*c*) The destruction by fire of all old stools after taking cuttings in January and February.

(*d*) Dipping of cuttings in nicotine 1 part to 800 parts water without soft soap.

The Ministry prevented the distribution of plants or cuttings from infested nurseries. In April 1928, when reappearance of the midge after its dormant period was anticipated, it was found that the insect had been stamped out in the Lea Valley.

It persisted upon only one nursery in Essex, and a single nursery in Bedfordshire was later found to be infested. By the following year the pest had been entirely eradicated and it has not been recorded since.

Thysanoptera

One of the most urgent problems at Cheshunt concerns insects commonly known as "Thrips". The species *Thrips tabaci* Lindeman, now well known as a vector of spotted wilt disease, fortunately not as yet prevalent in the Lea Valley, causes injury to flowers of various ornamental plants grown under glass, chiefly carnation, cyclamen and arum. No certain means of controlling the pest is at present available for the carnation grower. Investigations during the summer of 1935 have shown that the eggs are inserted into the tissues of the calyx, in the distal region, shortly before the carnation flower-bud opens. After the flower-bud breaks, additional eggs are laid in the petals, and the larvae hatching from those in the calyx creep into the flower.

When full grown the larvae escape from the open blooms, and the two pupal stages are passed in the soil or concealed in flowers. Pupae have not, however, been found amongst the leaves of the plant. It is of some interest to note that full-fed larvae floated upon water, and sometimes even when submerged in water will subsequently complete two moults and may even become adults under these uncongenial circumstances. As the foliage of carnation appears to be unpalatable to the larvae, it is only the flower which serves as a breeding place for the insect.

If these observations are correct, the broods of *Thrips* must be sent to market in flowers, as the larvae do not leave them for a considerable period of time after they have been cut. It is not improbable that many pupae or adults are subsequently distributed to nurseries in returnable boxes.

Further, it would appear that, in order to prevent the characteristic spotting of the petals due to the feeding of both adults and larvae, control measures should be directed towards the application of some deterrent which will prevent oviposition in the calyx, and deter the adults from entering the blooms after the flower begins to open.

With an insect so common upon outdoor plants, and one which passes a considerable period of its life in the soil, the chances of removing the source of infestation do not appear to be great.

Biological control of pests in greenhouses

The parasite *Encarsia formosa* Gahan of the greenhouse white fly, bred at the Cheshunt Experimental Station for distribution to growers, has reduced the white-fly population of the Lea Valley to such an extent that the grower no longer regards this insect as a pest in the district.

Efforts have been made to breed parasites and predators of other pests, but they have not been attended with the hoped-for success.

The remarkable life history of *Comedo opaculus* Thoms., an eulophid parasite of the tomato-moth caterpillar, has been fully studied, but this insect is ill-adapted to keeping its host in check.

Stethorus punctillum W.S., a ladybird beetle predatory upon red spider mite, was obtained from Belgium in May 1933, and was bred in large numbers for two generations, but the beetle was unable to adapt itself to conditions prevailing in tomato houses, shade such as is offered by vine foliage apparently being necessary for its reproduction. It is also doubtful if this predator or the cecidomyid, *Therodiplosis persicae* Kieff., can breed quickly enough to keep up with the generations of its host, which follow one another in such rapid succession during the summer months.

It is not likely that another parthenogenetically reproducing parasite such as *Encarsia formosa* will be met with just yet, but time spent in observation upon life histories adds to general knowledge of biological control, and at least helps in the selection of suitable parasites or predators which might be of economic value in the future.

III. SOME OBSERVATIONS ON THE COMPOSITION OF TOMATO PLANTS

By O. Owen, M.Sc., Ph.D., A.I.C.

The omission of potash or nitrogen from tomato fertilisers has pronounced effects on the quantity and quality of fruit and on the foliage of plants. In fact the condition of the fruit and foliage can often be an unmistakable indication of a deficiency in the soil of one of these nutrients. The addition of potash or nitrogen to soils deficient in these substances is always followed by a definite response in the plants—often of a speedy nature. In the case of phosphates, however, a different state of affairs exists. Successive crops have been grown in the same plots for some years without the addition of phosphates, and to all appearances the plants have been just as good as those receiving "complete" fertiliser. Nor have the weight or the quality of the fruit been affected. Further, a tomato crop rarely reacts to the addition of phosphates, although there was a fairly widespread belief among growers at one time that phosphates would "harden" tomato plants. It has to be remembered, however, that the plants are often propagated in soil which is fairly rich in phosphates, and it may be that almost sufficient phosphate is supplied in the early stages to carry the plant through its life history.

These facts prompted the study of the composition of the foliage of tomato plants and of whole plants, and some of the results obtained were discussed. Data for four different seasons were adduced to show that in the absence of added phosphates the potash content of tomato leaves was depressed—on the basis of percentage composition of dried material. In no case, however, was the potash deficiency accompanied by any of the symptoms associated with potash deficiency. The possibility that the water contents in the two cases might have been different was ruled out, as it was shown that differences of the same order persisted when the data were referred to a green weight basis. The hypothesis was advanced that in the absence of a certain concentration of phosphates in the soil tomato plants take up subnorma amounts of potash—the "normal" amount being that taken up by plants grown in soil dressed adequately with phosphates, potash and nitrogen. Further evidence on this point was supplied by analysis of foliage from plots receiving graded amounts of phosphate. A plot which had received no phosphates for some years was divided and the subplots received increasing amounts of phosphates—potash and nitrogen being kept constant throughout. Up to a phosphate content corresponding to about 11 per cent. $Ca_3(PO_4)_2$ in the fertiliser there was an increase in the percentage potash in the dried foliage.

Equally interesting results were provided by the analyses of plants growing in soil not receiving potash. In three out of four seasons the percentage of phosphoric acid is definitely increased by the omission of the potash. The effects of graded applications of potash to a "no potash" plot confirmed these findings. A potash concentration in the fertiliser of about 2·7 per cent. K_2O reduced the phosphoric acid concentration in the dried foliage to less than half that in foliage where no potash was applied.

These facts could be explained, in part, on the basis of the reduction of carbohydrate formation in the absence of potash. This would have the effect of tending to increase the percentage of other constituents. It is important to note, however, that the omission of potash actually reduces percentage of ash in the foliage, as will be seen from the following figures:

Percentage of ash in dried foliage

Complete artificials	32·53	26·97	34·47	30·40
Complete artificials without potash	31·32	25·36	27·57	25·01

In the first two years the reduction in ash content is negligible, but in the other two it is appreciable. In other words it may be said that the omission of potash increases the amount of what may be termed combustible material. In passing it may also be stated that the omission of potash has but little effect on the nitrogen content of the foliage.

Another explanation of the results is that the tomato plant is sufficiently adaptable to take up other nutrients, including phosphates, when there is a deficiency of potash. A survey of the literature indicates that these relations are not confined to the tomato plant.

The significance of these results lies in their application to the analysis of plants or foliage for the determination of the availability of soil potash and phosphates. Where a seedling method is used or the analysis of foliage is relied on, it is clear that care must be exercised in the interpretation of results. If by such methods a high available phosphate content is indicated, actually it may be indicative not only of adequate phosphate supplies but also of potash deficiencies. Also low potash availability might easily mean phosphate deficiency rather than a potash deficiency *per se.*

Analyses of whole plants at various times, considered in conjunction with manurial experiment and advisory experience, suggest that the need of phosphorus as a direct nutrient for tomatoes is low. It is calculated that a 40-ton crop removes about 670 lb. K_2O, 75 lb. P_2O_5, and 340 lb. nitrogen per acre, and these figures are probably in the region of the maximum annual removal of these nutrients.

Comparison of whole plants, manured and unmanured, gave the following interesting ratios:

	K_2O		P_2O_5		N
Manured	8·98	:	1	:	4·6
Unmanured	2·8	:	1	:	5·0

The ratio for manured plants is very interesting when it is recalled that in a "complete" tomato fertiliser widely used at one time the ratio is 0·62 : 1 : 0·51.

In the light of what has already been said the difference between the ratio K_2O/P_2O_5 for manured and unmanured plants is readily explained. Owing to successive cropping and the fact that the tomato plant requires relatively large amounts of potash, the potash level in the unmanured plot was low. Consequently there had been a growing tendency to an increased intake of phosphate. This depleted the phosphate reserves in the soil and consequently reduced the ability of the plant to make use of what little available potash there was. The two phenomena operating together result in a reduced potash intake and an increased phosphate intake. Hence part, at all events, of the fall from 8·98 to 2·8 in the K_2O/P_2O_5 ratio.

Another point of interest arose from the actual fruit produced by these two sets of plants. The manured plant gave 5·5 lb. per plant, while the unmanured one yielded only 3·2 lb. But the ratios fruit/green material in the two cases are:

	Manured	Unmanured
Fruit/green material	1·6	2·6

Now the grower obviously wants the ratio to be as high as possible, and it appears on the face of it that the unmanured plants have been functioning more efficiently than the manured ones. Actually from a commercial standpoint such is not the case. From the manured plot the proportion of first-grade quality fruit was 94 per cent. of the whole, from the unmanured plot it was only 43 per cent. This difference more than discounts the difference in the ratios fruit/green material.

IV. THE CONTROL OF RED SPIDER MITE AND TOMATO-LEAF MOULD

By W. H. Read, M.Sc., A.I.C.

The red spider mite (*Tetranychus telarius*), which growers term either spider or red spider, and tomato-leaf mould (*Cladosporium fulvum*), universally termed "tomato mildew", constitute two of the most serious enemies of the tomato grower. Few growers are not troubled with either of these, but the red spider mite is not at all particular in its diet, so that most horticulturists have experience of the damage it can cause under glass. I am not going to take up your time with a description of the red spider mite or tomato-leaf mould.

The history of the red spider mite has been dealt with by Lloyd, Speyer and Orchard, whilst Bewley and Small have dealt with tomato-leaf mould, and from their work it will be seen that the problem of controlling both of them is complicated by their over-wintering in the structure of the glasshouses. The spores of tomato-leaf mould are deposited in crevices and the like, whilst the red spider mite is adept at finding snug situations where neither sprays nor fumigants penetrate with ease. Another factor which adds to the difficulty is that the severity of the attack is greatest in both cases when marketable fruit is on the plants. This also applies to the attacks of red spider mite on cucumbers.

The presence of this fruit necessitates the use of sprays or fumigants which neither mark the fruit nor impart an unpleasant taste which does not disappear after a day or two. With regard to this tainting of the fruit it may be as well to state here that tomatoes acquire unpleasant flavours from fumigants much more readily than do cucumbers and retain them much longer.

Perhaps it will be as well if I now describe the methods which have been adopted for the control of the red spider mite before passing on to the control of tomato-leaf mould and joint control methods.

On occasions we have shown at Chelsea Flower Show plants attacked by the red spider mite, and many gardeners have sorrowfully shaken their heads and summed the cause up in two short words—"too dry", and gone on to say that all that is needed to stop it is to syringe the plants and keep the atmosphere moist. There is much truth in these remarks, for on certain plants such as peaches and many ornamental plants the pest can be kept in check in this manner. It would be difficult, however, to find many plants which are grown in such humid atmospheres as cucumbers, upon which the red spider mite is a very serious pest. Neither would it be advisable to suggest to a carnation grower that he damps his plants and continually maintains a humid atmosphere to rid himself of the red spider mite.

Consequently it is necessary to employ insecticides to keep the red spider mite in check. Many dusting and spraying methods have been tried in the past, but the first measure which gave anything approaching a satisfactory control was a flour paste liver of sulphur spray introduced by Lloyd. This was followed by a liver of sulphur soft soap spray which necessitated the use of soft water if clogging of the spraying plant was to be avoided, but when made with soft water was more easily prepared and marked the fruit to a less extent than the flour-paste mixture. Both these had a detrimental hardening effect on the plant—a minor disadvantage was the staining of the white-lead paint on the structure of the houses.

The introduction of petroleum-oil emulsion sprays from America gave growers a new weapon. Following chemical and entomological investigations at Cheshunt in 1927 a formula for the preparation of a suitable oil emulsion was published, and an emulsion corresponding to this formula is still used in considerable quantities:

Petroleum oil[1]	80 per cent. by vol.
Water	16 per cent. by vol.
Sodium caseinate	0·5 per cent. by wt.
Sodium coconut oil soap	1·7 per cent. by wt.
Ammonia (0·880)	1·5 per cent. by wt.

In many respects an emulsion of a highly refined petroleum oil constitutes an ideal insecticide, in that it need not contain any poisonous substance or anything which is odorous, easily noticed on the fruit or imparts a taste.

At first it was thought that emulsions of highly refined petroleum oils were without injury to glasshouse plants with a few exceptions, such as those with a "bloom" on the foliage or fruit, such as carnations and grapes.

Frequently tomato plants grown under modern glasshouse conditions are almost in an oedematous or dropsical condition in the spring at the time when the red spider mite is commencing to attack them. The application of an oil emulsion spray causes the disorder to appear by reducing the rate of transpiration from the leaves. Not only does the oedema seriously interfere with the normal growth of the plant, but the ruptured cells are liable to become infected with *Botrytis*.

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[1] Termed water-white petroleum and is free from sulphur and has an unsulphonated residue exceeding 95 per cent. Sp. gr. 0·866, 110 recs. Redwood No. 1 at 100° C.

On certain nurseries great attention has to be given to cultural details when oil-emulsion sprays have been applied early in the season, and in some cases it has proved too risky to spray with oil emulsions until the susceptible stage is passed. This seems to apply only to the tomato and possibly *Solanum capsicastrum* (winter cherry), and it is probable that later in the season many tomato crops benefit from the application of oil emulsions, especially plants which are ageing.

All proprietary emulsions do not possess this tendency to cause oedema to the same extent, and investigations are in progress to determine the factors responsible. As a result of the danger of oedema a very large number of substances have been tried as spray mixtures to replace oil-emulsion sprays. Emulsified glyceride oils were tried but not found as satisfactory as petroleum oils, whilst emulsified synthetic solvents, neutral tar oils and solutions of naphthalene in various solvents all caused very severe injury in concentrations which killed the active stages of the mite only. At one time a proprietary product said to consist mainly of sulphonated oxidised petroleum hydrocarbons appeared to be the solution, for it gave an excellent control of the red spider mite at a dilution of 1 in 300. Comparative tests showed that it had at this dilution a greater tendency to cause oedema than petroleum-oil emulsions used at a dilution of 1 in 100. From time to time derris extract sprays containing this or similar substances are submitted for trial, and it is as well to bear in mind this tendency for causing oedema.

From trials of numerous sprays as substitutes for petroleum-oil emulsions it would seem that derris extracts are of most promise. Both emulsified extracts of derris and sprays prepared by incorporating a wetting agent with finely ground derris have been tried. Our experiments this year have shown that there is some factor which reduces the effectiveness of these sprays in the summer, but where there is risk of oedema in the early part of the year such sprays are worthy of trial.

A brief outline of the methods of controlling the red spider mite on other important glasshouse crops may be of interest. In the case of the cucumber no trouble is experienced with the application of oil emulsions apart from the ever-present difficulty of getting the spray to reach all parts of the foliage—a matter on which I hope to say a few words in connection with the use of combined insecticide-fungicide mixtures. In addition to the disadvantage of expense, many derris extract sprays have a slight hardening effect on cucumbers, and the only satisfactory alternative to spraying with a petroleum-oil emulsion is to fumigate with naphthalene. The method of fumigating with naphthalene is now used only in cases of very severe infestations and towards the end of the season. For this purpose a highly refined naphthalene is employed, and the vapour obtained by heating this in what are now termed naphthalene lamps or by the broadcast method in which the naphthalene is scattered evenly on the surface of the soil and path of the glasshouse. The fumigation of cucumbers by the "broadcast" method devised by Speyer is adequately described in our annual reports for 1924 and 1925, and it will be seen that high temperatures (74° F.) must be maintained over long periods—at least 12 hours—in order to obtain a satisfactory control of the red spider mite. If the necessary high temperature can be maintained sufficiently long the process is very effective. The fumigation of tomatoes by this method is impossible, for even if lamps are employed to obtain the necessary concentration of naphthalene vapour the damage to the plants and tainting of the fruit renders the process useless from a commercial standpoint.

The red spider mite is a very serious pest of the carnation also, and as previously mentioned oil-emulsion sprays cannot be used on this crop. Liver of sulphur and soft soap was at one time the standard recommendation, but this does not find favour on account of the marking of the bloom and hardening of the plants. The use of derris sprays has been investigated and these are now extensively used, but the most promising control measure is naphthalene fumigation.

Recent experiments at Cheshunt have shown the impossibility of fumigating carnation houses with naphthalene by the "broadcast" method if plant injury is to be avoided except under very favourable weather conditions. The carnation is susceptible to injury by continued high temperatures. A great part of the time during which high temperatures must be maintained in the "broadcast" method of fumigation is employed in obtaining a sufficiently high concentration of naphthalene vapour, whereas when lamps are employed the concentration can be built up quickly, is more under the control of the operator and the fumigation can be conducted at a lower temperature.

A tremendous number of other substances have been tried as fumigants, many of which destroyed the mite at low concentrations, but generally speaking their toxic effect on the plants corresponds with their effect on the mite.

I will now deal with the measures which can be adopted at the end of the growing season. The investigations conducted on numerous tomato and cucumber nurseries by Orchard show that it is necessary to undertake these measures before the majority of the mites have left the plants and found hibernating places. Fumigation with naphthalene (1 lb. per 1000 cu. ft.) is valuable if lamps are available or high temperatures can be obtained by applying pipe heat to augment sun heat during favourable weather conditions. For this purpose a cruder naphthalene can be employed, since scorching of the plants is advantageous rather than otherwise. As an alternative sulphur may be burnt—a dose of 1 lb. per 1000 cu. ft. being required.

The burning of sulphur to cleanse glasshouses is an old practice and has the advantage of destroying fungus spores also. It has a great disadvantage, however, in that damage may be caused to following crops. As a result of the investigation by Orchard of a case of severe injury to a crop of chrysanthemums, samples of the damaged foliage were analysed and considerable quantities of zinc discovered. This was found due to the leaching by condensed moisture of zinc sulphate from the zinc white paint of the house, this salt having resulted from the action of sulphur dioxide on the zinc oxide and subsequent oxidation. In other cases zinc sulphate has been formed from the galvanising of supporting wires, and whilst it is easy to remove the zinc sulphate from these by washing, it is more difficult to remove it from the paint, in addition to which the paint film must obviously be damaged.

Consequently burning sulphur is only advised where leaf-mould or mildew has been present as well as red spider and the paint of the house is free from zinc. Washing down the interior of the houses with formaldehyde or emulsified cresylic acid solutions kills a number of the exposed mites—mainly mechanically, but the vapour of neither of these substances destroys many of those hibernating in crevices. As I have stated before, the value of end of season measures is greatly reduced if delayed until the majority of the mites have hibernated, and in the majority of cases many of the mites have left by the time the crop has finished.

I will now deal with tomato-leaf mould. The control of tomato-leaf mould and the red spider mite are now interconnected in many instances.

Whilst the maintenance of low atmospheric humidities checks the growth of tomato mildew the requisite conditions for this purpose are exceedingly difficult to maintain when nature wills otherwise, and chemical control measures remain of great importance.

A great number of fungicides were tried, including dusting with sulphur, vaporising sulphur with special fumigating apparatus or by putting sulphur on the heated pipes, and spraying with lime sulphur or liver of sulphur. None of these were particularly effective. Small tried a number of fungicides in conjunction with some of the modern synthetic wetting agents, which were found very effective in obtaining good wetting of the spore masses, and in 1930 suggested the use of ammonium copper carbonate solutions. Unfortunately this caused damage when tried on nurseries in 1931 and in consequence other attempts were made to obtain a safer spray fluid.

Bewley and Orchard reinvestigated the fungicidal properties of the sodium salt of salicylanilide, and finding this caused severe damage to tomato plants at effective fungicidal concentrations tried salicylanilide itself. They found a suspension of this very effective when used in combination with a proprietary wetting agent—Agral 1. Salicylanilide suspended to form a paste containing 50 per cent. of the active material is known by the trade name Shirlan H.B.—the name deriving its origin from the Shirley Institute where its fungicidal properties were first investigated. The mixture recommended by Bewley and Orchard consisted of ⅛ fluid oz. of Shirlan paste and ¼ oz. of Agral 1 to 1 gallon of water, and this gave good control of tomato-leaf mould with but slight marking of the fruit where drops collected at the blossom end. Soon after a more effective wetting agent, Agral N, was introduced, and this was substituted for Agral 1 and half the quantity, *i.e.* ⅛ oz. per gallon employed with consequent diminution of the marking of the fruit. Other synthetic wetting agents have been tried and found equally effective, although very few proprietary wetting agents are effective at the concentration recommended by the makers.

This mixture of Shirlan and Agral N has also proved effective in the control of cucumber mildew, *Erysiphe cichoracearum*, and mildew of the chrysanthemum, *Oidium chrysanthemi*.

Unfortunately mildew and red spider very often occur together, and frequent sprayings are required to keep them under control. It was found that damage resulted when oil-emulsion sprays and the Shirlan mixture were applied within 10–14 days of one another, and since sprayings to control either the red spider mite or tomato-leaf mildew require to be applied as often as once a fortnight, it is seen that other control measures for one of the two had to be found.

The reason for the damage appears to be that salicylanilide is slightly soluble in petroleum oil, and the oil penetrates the tissues, taking with it salicylanilide which causes injury. The same trouble has been experienced, although to a less extent, when emulsified solutions of derris extract have been applied shortly after applications of Shirlan.

Previously a proprietary copper oxychloride suspension containing about 17 per cent. of copper had been tried in conjunction with a wetting agent as a control for tomato and cucumber mildews. This was found an effective fungicide, but marked both cucumber and tomato fruits rather badly and also hardened the plants.

Since petroleum-oil emulsion sprays have a softening effect on plants, a combination of this oxychloride mixture and our standard oil emulsion was tried, and it was found that not only was the hardening effect on the plants eliminated but the marking of the fruit was much less and now unimportant. Furthermore, the fungicidal action of the copper compound was increased. This is probably partly due to the slight fungicidal effect known to be possessed by petroleum-oil emulsions, but mainly to the increased retention of the copper oxychloride by the leaves.

Other samples of copper oxychloride have been tried recently, but so far none of these has proved quite as effective as the proprietary suspension. How far this is due to differences in chemical constitution or to differences in particle size cannot be decided until further work has been undertaken, but in all cases a better control has been obtained where they have been used admixed with a petroleum-oil emulsion.

So far all the petroleum-oil emulsions tried have given good results when used at the normal dilution recommended for the control of red spider with the addition of ½ fluid oz. per gallon of the copper oxychloride suspensions, but the possibility of oil emulsions containing substances which will render sufficient copper soluble to cause injury must not be overlooked.

In connection with the application of these insecticide-fungicide mixtures it has been found that the best control is obtained where the spray is applied at really high pressure, up to 200 lb. per square inch, using a medium-sized nozzle aperture. Furthermore the amount of spray fluid required to obtain efficient covering of the foliage is less than where low pressures and fine nozzle apertures are used.

Many other substances have been tried in a combined insecticide and fungicide spray, for it is obvious that the ability to apply both insecticide and fungicide in one operation is of great value. Cuprous cyanide has been tried in combination with oil emulsions and found but slightly less effective than the copper oxychloride powders.

Since all these mixtures leave slight deposits on the fruit, experiments have been made with oil-soluble substances. Emulsified 50 per cent. solutions of copper oleate in cotton-seed oil which had given promising results in experiments on the control of fungus diseases of the rose were tried but caused too much damage, in addition to which red spider mite was not killed. Cotton-seed oil emulsions were not effective at usable concentrations, and emulsified solutions of copper salts in petroleum oils caused injury and did not kill red spider mite, probably on account of the high viscosity of such solutions.

Petroleum-oil solutions of sulphur caused extremely severe injury in spite of the low solubility of sulphur in petroleum.

A sample of copper naphthanate containing 12 per cent. of copper was also tried in solution in petroleum and glyceride oils, but again damage was not caused, although in this case solutions containing 20 per cent. of the copper compound had but very slightly greater viscosities than the oils themselves.

From these tests with oil-soluble copper compounds it seems unlikely that petroleum oil-soluble compounds can be safely employed.

Samples of the copper salt of salicylanilide were tried in combination with oil emulsions, but here again damage was caused. This was found due to the presence of salicylanilide in the mixture, for the copper salt is insoluble in the petroleum oil used. Samples free from salicylanilide are being prepared for further trials.

Two devices which render the vaporisation of sulphur much safer and enable larger amounts of sulphur to be vaporised than previously have been tried recently, but although the mildew is checked by sulphur vaporisation the control obtained is not good and the plants are slightly damaged. Small tried a large number of substances as fumigants for the control of tomato-leaf mould, and quinone, the most promising of these, has been tried on a larger scale. Quinone, however, has a low vapour pressure and condenses in minute crystals when evaporated from lamps which prevent good distribution. Also it has a strong flavour which is readily imparted to tomatoes.

V. SOME IMPORTANT DISEASES OF GLASSHOUSE PLANTS

By P. H. Williams, B.Sc.

The vast majority of the enquiries dealt with at the station in the course of a year are concerned with tomatoes and cucumbers. I shall therefore confine myself to diseases of those crops.

Before discussing any of the problems in detail, however, I should like to point out that a particular disease may vary in importance from year to year. Before the War, tomato canker caused by *Didymella lycopersici* was so widespread as to threaten the existence of the tomato-growing industry. Nowadays it is of minor importance, although a few cases occur each year. Even more striking is the way in which the cucumber leaf spots, caused by *Colletotrichum* and *Cercospora* have died out. In 1919 the former was of primary importance; now it is so rare that I have never seen a specimen. In this case the adoption of a resistant variety, Butchers' Disease Resister, was the main cause of the disappearance of these diseases, in conjunction with more thorough winter cleaning and the increasing use of sterilisation. No doubt the same practices have helped to reduce the severity of tomato canker.

On the other hand, several diseases have become more important in the last few years. We used to congratulate ourselves that tomato foot rot caused by *Rhizoctonia solani* was found only in Guernsey and not in this country. Now it is quite common and sometimes causes severe losses. Virus diseases, too, have become more widespread.

In recent years various forms of root rot have become very common in the tomato. Early in the year the roots of young pot plants are often brown with some decay and the plants themselves are stunted and "blue". Very frequently the club-shaped pores of *Thielaviopsis basicola* are present in the brown tissue. Some years ago I was able to show that this fungus is able to cause appreciable damage only when conditions are unfavourable to the growth of the plant. Thus periods of dull, cold weather in the early part of the year are especially favourable to the development of this disease and if they persist the plants may die. Usually, however, the advent of brighter weather will enable the plants to grow away from the fungus which cannot keep pace with the production of new roots. Prevention is better than cure, however, and the use of sterilised soil will eliminate this disease.

After planting out, plants may remain stunted and hard, and in such cases examination shows that most of the roots are completely rotten. In many cases there are depressed areas at the base of the stem indicating an abortive attack of foot rot. From the stem and roots we can isolate *Phytophthora* sp., most frequently *P. parasitica*. In these cases, the use of Cheshunt compound when replacing diseased plants is usually of value, but sterilisation of the soil in the following winter may be necessary in serious cases.

Later in the summer, generally about July, we begin to meet with root rot caused by *Colletotrichum atramentarium*. In this disease the cortex of the root is destroyed so that the outer layers slough off. The fungus also invades the wood, and the characteristic sclerotia are formed both in the wood and the bark of the root. Frequently it penetrates the stem from the root causing a dark brown discoloration of the wood. Soil sterilisation is the remedy.

More difficult to diagnose and treat are those cases where no definite parasite can be demonstrated, indeed it is often impossible to determine the cause of the trouble from examination of the specimens alone. Inspection of the crop and discussion with the grower are usually necessary before coming to any conclusion. In most cases the primary cause seems to be cultural.

In the early part of the season, planting in cold soil will give the plants a check from which they take a long time to recover. The roots are killed or so weakened as to fall a prey to parasitic fungi. Where the soil is open and well drained the plants will gradually grow away, but in heavy, compact soils which are deficient in opening materials and remain cold and wet, the roots may never recover and the plants will flag and die as soon as the hot weather comes. In such heavy soils, too, incautious watering may lead to waterlogging with consequent suffocation of the roots.

Remedial measures consist in the use of opening materials such as straw and peat. Straw, placed vertically against the side of the trench during trenching, seems particularly good by providing channels through which air and water may reach the lower layers of the soil. Periodic steaming is also valuable, not only by destroying parasitic fungi, but by altering the physical and chemical properties of the soil. During the growing season, the plants may be encouraged to make fresh roots at the collar by mulching with straw manure or peat. Soil may also be used, but, if virgin turf is employed, there is a risk of wireworm in the following year.

Before leaving the subject of tomato roots, I may mention a peculiar condition which is common; indeed it is found on almost every plant at the end of the season. The roots become swollen and the outer layers become corky and split. The length of root involved varies from half an inch to more than a foot. The cause of this condition remains a mystery. Some years ago I isolated a number of fungi and bacteria from such roots. A few were well-known parasites such as *Fusarium culmorum*, but the pathogenicity of the remainder was very doubtful. Direct inoculations were unsatisfactory because of the difficulty of finding suitable roots on plants growing in pots, while the impossibility of keeping the soil free from contamination in experiments lasting several months ruled out soil inoculations. Inoculation of roots growing in the border seems to be the only method likely to lead to conclusive results, but up to the present this has not been possible.

With cucumber roots we have very much the same story as with the tomato. There is a definite disease caused by species of *Fusarium*, but in a very large number of cases rotting of the tap root and side roots may be started by unsuitable soil conditions. The cucumber crop leads to the production of some substance in the soil which is toxic to subsequent crops of cucumbers. This toxic substance is washed down into the base and accumulates there so that in time it becomes impossible to grow a satisfactory crop, for as the roots reach the base they die and start to rot. The rot spreads upwards, and even if the plant does not die it is crippled and the crop suffers, a large number of the fruit "damping off" or becoming "crooks". The retention of the toxin is worst on heavy soils, and some improvement may be obtained by replacing the base with a gravel soil from which the toxin may be washed out, in part at least, by flooding. Steaming removes the contamination and is the only real remedy. Formaldehyde and cresylic acid produce little benefit, showing that it is not a question of living organisms but of some chemical substance which is broken down by heat.

Beds which are compact and ill-drained are also a common source of trouble. Whether the roots die from some purely physiological cause or are attacked in their weakened state by fungi and bacteria is still obscure. Improvement may often be obtained by resting the plants for a short period. The beds are allowed to dry out for a week or 10 days and the house ventilated while the foliage is maintained by damping. Then the plants are trimmed, the beds top dressed with a suitable compost and normal culture resumed.

Another important group of diseases are those caused by viruses. The number of these is constantly growing. Fortunately, not all occur in this country, and of those that do only three are of commercial importance.

From the growers' point of view the most important tomato virus diseases are streak and yellow mosaic, since both mark the fruit and make it unsaleable except as "roughs". Ordinary mosaic can cripple the plant and usually lowers the crop, but since the fruit is not marked we do not regard it as so serious as the other two diseases. Mixed virus streak also occurs occasionally, and when it does may be very serious.

Spotted wilt has received a lot of attention, principally because of its wide host range. It is difficult to decide whether it really is as important as some workers would suggest. Certainly, where only tomatoes are grown, the grower need not fear it. Most of our specimens come from private gardens and mixed nurseries. Where tomatoes are grown as a late crop in houses used for raising bedding plants, there is undoubtedly a risk of infection if the tomatoes are planted out before the other plants have been cleared off. By keeping the tomatoes back a week or two, and fumigating to destroy *Thrips* and other insects in the houses before planting, growers who have suffered losses from this cause have been able to prevent a recurrence.

In combating these virus diseases, the importance of using seed from clean plants must be obvious. In streak, for example, the virus occurs in the seed coat and may quite easily be conveyed to a certain number of plants in the operations of pricking out and potting. Carrier plants are also known in certain of these diseases, and therefore the absence of infected seedlings does not mean that the disease is not present, for it may be latent and show itself only later.

Another source of infection which has been demonstrated recently is the tobacco in the commoner brands of cigarettes. Whether it is possible to make any use of this fact is another matter, for it is difficult to prohibit smoking by the workers. No doubt, however, some will take it to heart with benefit to their employers.

If infected plants are noticed in the early stages of growth, they should be pulled out and replaced, or shoots from neighbouring plants may be trained up in their places. Often, however, they remain unnoticed and consequently a large number of plants become infected. In these circumstances roguing out is not possible and we must rely on keeping the plants growing as vigorously as possible. Attempts may also be made to isolate infected plants by leaving them to the last when trimming, but success depends to a large extent on the individual worker. Infected plants should not be "stopped". This is important, for not only may the disease be spread in the process of "stopping" but the shoot which grows out is usually weak in a diseased plant and does not bear much fruit.

In the cucumber there are three virus diseases. Of these, green-mottle mosaic, though the commonest, does not do a lot of damage. The other two, yellow mosaic and yellow-mottle mosaic, mark the fruit and therefore are more serious when they occur. The evidence shows that the marking is most severe at high temperatures, and something can be done to diminish losses by keeping the temperature down. With yellow-mottle mosaic, there is also the risk that tomatoes may be infected with the production of fern leaf.

Control measures as with the tomato consist of roguing and cultivation to promote a free growth. Seed transmission is important and at Cheshunt we were able to free our cucumber stock from yellow mosaic by roguing out all diseased plants and saving seed only from plants which had remained healthy throughout the season. Now seed is only saved from plants grown in a special house, and no virus disease has occurred from some years in our main cucumber crop.

On some nurseries and even in particular houses on a nursery *Botrytis* may cause a lot of damage. In some cases it is almost impossible even to break off a leaf without *Botrytis* growing on the ragged edges of the wound and thence penetrating into the stem. Too high humidities, especially with a drop in temperature at night leading to deposition of water on the plant, seem to be the principal factors favouring infection. Adequate ventilation will do a lot to prevent it, and so will careful pruning. Diseased parts of the stem should be cut out and the exposed tissue rubbed with a piece of liver of sulphur or covered with a paste of the same substance.

On the cucumber, the dead flowers which remain on the end of the fruit may act as a focus of infection. In one case which occurred this year, where infected flowers fell on to a leaf, a necrotic spot developed. In another case the fungus grew backwards into the fruit. In the first case, spraying with a colloidal copper compound was used successfully, for the grower was unable to maintain a sufficiently high night temperature to prevent a "sweating".

Various disorders of the fruit both of tomatoes and cucumbers may also be serious. I have already mentioned "damping off" of the cucumber fruit and the production of "crooks" as being associated with poor root action. In the tomato we have a number of different conditions due to manurial deficiencies and other causes. Blotchy ripening follows an unbalanced growth due in most cases to a lack of potash, although shortage of nitrogen may sometimes be the cause. Somewhat similar is greenback, in which the tissue around the calyx remains hard and green when the rest of the fruit is ripe. This is due to overheating by the sun when the plant is short of potash, and often appears when too many leaves have been removed. There are also various forms of internal necrosis which seem to be related to the well-known blossom end rot. They are usually due to a temporary shortage of water during the time before real watering commences and so are most common on the bottom trusses, but it seems that any check in the steady growth of the fruit may produce this condition.

REVIEWS

Die Fusarien. By H. W. Wollenweber and O. A. Reinking. Pp. viii + 355, with 95 text-figures and illustrations. Berlin: Paul Parey. 1935. Rm. 15.

It may be said at once that the joint labours of Dr Wollenweber and Dr Reinking have given to mycologists and plant pathologists an authoritative treatment of first importance concerning *Fusarium* and *Fusarium* diseases. *Die Fusarien* marks the end of that long period during which this subject has been in a state of such hopeless confusion that few mycologists cared to tackle any aspect of it except in a most general way. No longer is it permissible for an observer to describe a species of *Fusarium* as he sees it, and, unable to find a corresponding description in the mass of scattered records, to apply a new specific name and so make confusion worse confounded.

The work comprises two main parts: *Fusarium-Systematik*, obviously the work of Wollenweber; and *Fusarium-Pathologie*, equally obviously the work of Reinking mainly. A third section of 21 pages covers the *Fusarium-Synonymie und -Homonymie*, the volume being completed by a fully annotated index.

The section *Fusarium-Systematik* shows the masterly handling which comes of many years of progressive work on the subject. The author has made it his special study, since, in association with Dr O. Appel, order began to emerge from chaos when the well-known *Monographie der Gattung Fusarium* was published in 1910. In 1931 Wollenweber published the *Fusarium-Monographie*, the first and only work of its kind, one primary object in which was the elimination of redundant specific names and synonyms. The successful development of this plan is demonstrated in the third section of *Die Fusarien*, which gives a list of more than 1000 synonyms, together with the modern rendering according to Wollenweber's simplified nomenclature which reduces the group to sixty-five species, with fifty-five varieties and twenty-two subsidiary forms. This itself appears to be sufficiently formidable, but is as a flood of light in comparison with the gloom of the former so-called 1200 odd species and unlimited descriptions. The form of presentation of these synonyms is simpler and clearer than that adopted in *Fusarium-Monographie* where such collected information was first published.

In fact, this first section, *Fusarium-Systematik*, is a revised and condensed version of *Fusarium-Monographie*, with the addition of line-drawn figures illustrating the typical and ascigerous forms of most species, and keys to the groups and subgroups of the species. These aids, together with the morphological data, will go far to assist the general mycologist to identify species encountered in diseased plants. Even so, identification may be neither simple nor speedy without very considerable experience. This arises from the fact that in nature the environmental conditions are variable, and in *Fusarium* species there is more morphological variability arising from environmental differences than is to be found in most other fungi. If the investigator carries the organism into artificial cultures, very considerable variations may be induced according to the nature of the substratum. The variations may be of such number and magnitude as to render experience the invaluable factor in identification. It is in no sense an adverse criticism to point out that *Die Fusarien* gives no assistance in these respects; not one volume but many would be required to deal with such aspects, all of which goes to show that identification of difficult or variable forms still remains for the experienced specialist. However, as a concise, well-illustrated, and perfectly systematised view of the species of *Fusarium*, this part of *Die Fusarien* is likely to remain the standard work for many years to come.

The second main part of this work, *Fusarium-Pathologie*, is a new departure in the literary presentation of *Fusarium* diseases, though similar in plan to a number of American publications. Plants are named in alphabetical order, and for each is given a description of the disease or diseases caused therein by *Fusarium*, together with a bibliography of publications bearing on these diseases. That such an extensive range of investigations and results should be summarily written in 174 pages, and compiled up to 1934 in a work published in 1935, is a tribute to the care and labour that the authors have devoted to this publication. Plant pathologists in general have long considered that few or none of the species of *Fusarium* show any marked specialisation toward host plants, and that the group as a whole comprises forms parasitic on a very wide range of vegetation. The view is amply substantiated by this collection of *Fusarium* diseases under the heading of host plants. The latter range from algae and fungi to coniferous and broad-leaved trees, including plants of economic importance and ornamental value, from temperate to tropical regions. The authors deal with more than a hundred kinds of plants on the lines mentioned; of these, Brassicae, *Gossypium*, *Lycopersicum*, *Musa*, *Solanum* and *Triticum* are dealt with rather more fully than others. Brief mention is made also of *Fusarium* diseases of animals, the hosts ranging from nematodes to mammals, and to *Fusarium* in dairy products, water, sewage, etc. The verbal descriptions are exemplified by nearly fifty well-produced photographic illustrations, mainly culled from original investigations.

Control measures such as warm water treatment of seeds, the use of mercurial fungicides, and resistant varieties are mentioned where appropriate but not described in detail. Knowledge of this aspect is but fragmentary, and many years of investigation work will be required to make it anything like complete for a group of diseases of such extensive range and complexity.

In the *Fusarium-Pathologie* section causal species of the diseases are named. As is so frequently encountered in practice, several species may be involved separately or together in a disease; to quote a simple instance, the authors describe for *Dianthus*: "Wilt (*F. dianthi*); foot or stem rot (*F. culmorum, avenaceum, sporotrichioides, scirpi v. acuminatum*, etc.); bud rot (*F. poae, reticulatum, avenaceum, tricinctum*, etc.)." The advantage of having the morphological characters of species so accessible in the first section of the book is obvious. As mentioned above, the plant pathologist will still have to overcome certain difficulties as regards identification.

To sum up, if plant pathologists will recognise the fact that there is no "royal road" to the full elucidation of *Fusarium* problems, they will the better appreciate such a well-ordered guide as is provided by *Die Fusarien*. It is a guide to the solution of problems, produced with scientific accuracy by workers who have had special experience of them, and no up-to-date mycologist or technical plant pathologist may omit it from his library. In fact he should thank the authors for giving him the benefit in such useful form of so many years of unremitting toil and care.

F. T. BENNETT.

Biological Processes in Tropical Soils. By A. Steven Corbet. Pp. xiv+156, with 16 Plates and 10 text-figures. Cambridge: W. Heffer and Sons, Ltd. 1935. *7s. 6d.*

Dr Corbet's book has special reference to rubber-plantation areas in Malaysia where the author was sometime Bacteriologist to the Rubber Research Institute. It contains chapters dealing with: I, the geography, geology and climatology of Malaysia; II, the plant life of Malaysia; III, the soil fauna; IV, soil micro-organisms; V, the bacterial growth curve; VI, the soil organic matter; VII, the nitrogen cycle; VIII, Jenny's Law; and IX, some practical considerations, such as the burning of cleared land, leguminous cover plants, natural covers, the effect of fertilisers on the soil, and garden soils. Each chapter is summarised and followed by references. There is an Appendix containing methods for soil examination, classification of bacteria and fungi, simple conversion factors and a brief bibliography. The book closes with author and subject indexes, an index to animals and an index to plants.

This little book covers a very wide field, and not only is it an interesting attempt to apply modern soil-microbiological research to the problems of a wet tropical area, but it also contains valuable and suggestive material. On the other hand, the book is unequally balanced, much of the content is elementary or inadequate and, in many places, the scientific foundations will hardly bear the superstructure the author builds upon them. It is a great pity that the author did not submit his manuscript before publication to workers with special experience in certain of the more scientific problems he considers.

WILLIAM B. BRIERLEY.

Problems in Soil Microbiology. By D. WARD CUTLER and LETTICE M. CRUMP. Pp. vi + 104, with a map, 18 text-figures and 32 tables. London: Longmans, Green and Co. 1935. 9*s*.

This book, which incorporates the substance of a series of lectures given at Aberystwyth, is based on researches carried out in the General Microbiology Department of the Rothamsted Experimental Station by the authors and their colleagues. As the authors state in their preface, it is in no sense a text-book of soil microbiology and its title is somewhat misleading in that it gives the impression that the book covers a much wider field than is actually the case. A more accurate title would have been "Some Problems of Soil Bacteria and Protozoa."

Chapter I is introductory and is a very general consideration of the soil as a suitable home for micro-organisms. Chapter II is devoted to the bacterial population under field conditions, emphasis being laid on bacterial adaptability and versatility. In Chapter III the relation of bacteria to nitrite is discussed and attention is drawn to the fact that numerous bacteria are able to interfere with the nitrite part of the so-called nitrogen cycle. Chapter IV deals with carbon dioxide production by soil and the difficulty of classifying bacteria by their behaviour on carbohydrates. Chapter V contains an account of the growth and encystment of protozoa under pure cultural conditions in relation to physical factors and food supplies. In Chapter VI the same problems are discussed but in relation to protozoa in the soil, and there are some interesting speculations on the vital rhythms shown by soil micro-organisms. Chapter VII is devoted to a consideration of the interactions between the soil organisms and, apart from the bare inclusion of the word "fungi" on p. 7, this is the only chapter in which the fungi and algae are even mentioned. The book closes with a "list of literature cited" which shows several interesting features: of the fifty references twenty-seven are Rothamsted publications, no citation is taken from *Soil Science*, and there is no mention of the work or names of Waksman and his associates. It must have been very difficult to write a book on "Problems in Soil Microbiology" without mentioning Waksman. The book is simply and clearly written and is a record of personal researches which any Institution might well be proud to father. There is, however, the grave danger that readers lacking a background of a more general knowledge of soil microbiology may acquire a distorted perspective, namely, that protozoa are really important soil organisms, followed by bacteria and, in the dim distance, by fungi and algae. Even in their final chapter the authors do practically nothing to suggest a more balanced perspective.

The book is one of the Rothamsted Monographs on Agricultural Science and its price seems rather high.

WILLIAM B. BRIERLEY.

Limnology. By P. S. WELCH. Pp. xiv + 471, with 46 figures and 53 tables. London: McGraw-Hill Publishing Co., Ltd. 1935. 30*s*.

Owing to our comparative lack of extensive inland waters "limnology", as a branch of science, has received little attention in Great Britain. A certain amount of research is carried out under the aegis of the Ministry of Agriculture and Fisheries in relation to fresh-water fisheries, and under the Department of Scientific and Industrial Research in relation to water-pollution by industrial effluents. Valuable knowledge has also accrued from the investigation of Lake Windermere. The six-volume *Bathymetrical Survey of the Scottish Fresh-water Lochs* issued by Sir John Murray and his associates in 1910 is, of course, a landmark in the science but, since that date, practically the only book on the subject published in this country is Carpenter's *Life in Inland Waters*, 1928, and this deals almost entirely with the fresh-water fauna. In the U.S.A. and in European continental countries, more especially of recent years in the U.S.S.R., where inland waters are of great importance, limnology is in a highly active condition. In Russia alone there are over thirty stations contributing to hydrobiological research. Good general treatises in English have been conspicuously lacking and, although in the U.S.A. there have been published Ward and Whipple's *Fresh Water Biology*, 1918, and Needham and Lloyd's *The Life of Inland Waters*, 1930, a text-book of "limnology" has not so far been available. The present volume has, therefore, something of the nature of a pioneering venture in which the author has had to face the difficulties inherent in the composition of a general text-book in a new and largely uncharted territory.

The work is divided into five parts. Part I is preliminary to the rest of the book and deals with various introductory matters; the extent, distribution and dynamics of standing and running waters; and the origin and diversity of lakes. Part II commences the more serious portion of the book, and is devoted to a detailed consideration of the physical and chemical conditions of fresh-water environments. It is the best short survey of this subject I know. Part III commences the biological portion of the book and contains chapters on the influence of physical and chemical conditions; the organisms found; plankton; bacteria, fungi and non-plankton algae; higher aquatic plants and their limnological significance; nekton; benthos; and a final interesting chapter on biological productivity. Part IV is devoted to special types of standing-water environments, such as ponds and bog lakes, whilst Part V deals with running-water environments.

It will be seen that the author has used the term "limnology" in its widest sense and, although he gives far more attention to standing than to running waters, this balance merely reflects the present state of knowledge. The author's approach is through a study of the physical and chemical factors of fresh-water environments, which are reviewed and given a limnological bearing before the biological phenomena which involve them are considered, and in his treatment he assumes a knowledge of the elements of physics, chemistry, botany and zoology as a background in the reader's mind. He confines himself to a consideration of data and principles and proposes to deal with limnological methods and practices in a further volume now in preparation.

The book is simply and clearly written, and the author has collected from widely scattered sources an immense number of data which he marshals in logical sequence. The work is definitely stronger on the physico-chemical side than on the biological side, and it is weakest in its discussion of floristic aspects. Without illustrating by extensive quotation, one may point to Fig. 38 where "N-fixing molds" and "N-fixing algae" are given equal place with "N-fixing bacteria" in the nitrogen cycle, or to Chapter X where bacteria are stated to be fungi.

A valuable feature of the work is the classified bibliography, running to fifty-five pages, which, although not complete, is a splendid guide to the modern literature on the subject. One may perhaps hope that when a second edition of the work is called for, the author will strengthen and amplify his treatment of the more purely biological aspects of limnology and also take more note of work in soil, effluent and industrial microbiology and microbial physiology which has important relationships to life in inland waters. One must not, however, look a gift-horse too hard in the mouth. In so far as it goes the book is a notable achievement, and Prof. Welch is to be congratulated on the production of a volume which must have a lasting influence on the development of the science.

WILLIAM B. BRIERLEY.

Plant Viruses. By KENNETH M. SMITH. Pp. 107. London: Methuen and Co., Ltd. 1935. 3*s*. 6*d*.

The author states that the aim of this book "is to bring to the notice of workers in other branches of science, and particularly botanists and entomologists, some of the more interesting and important facts of the study of plant viruses. In their study lie problems of fundamental biological importance, such as their affinities with enzymes, their intimate relationship with the living cell, their curious and interesting association with insects, and finally the possibility that they may represent a new and simple form of life". The book is a suggestive summary of modern research into virus problems and should fulfil the author's aim.

The book opens with an introductory chapter dealing with the history and characteristics of plant virus diseases with comments on the chief diseases in various countries, their increase and economic importance. Chapter II, on the technique of plant virus study, gives brief details of glasshouse technique, methods of experimental transmission, the culturing of insect vectors and the separation of viruses in a disease complex. The constructional details of insect proofing of glasshouses and notes on grafting methods seem out of place in such an essay as this. Chapter III describes natural modes of transmission by seed, soil, vegetative propagation of infected parts, and by insects, and discusses the factors governing spread in the field. The chapter closes with a very brief account of Smith and Bald's startling work on tobacco necrosis, which opens up new avenues in virus study. Chapter IV discusses problems relating to the virus in the host such as symptoms and x-bodies, local lesions, and the controversial subject of virus movement. In Chapter V the virus outside the plant is considered, including an account of physical properties, tissue culture, particle-size of viruses and purification of virus suspensions. Here again the detailed formula on p. 56 seems out of place; it in no way contributes to the argument and any worker requiring it would consult original papers. Chapter VI is devoted to the virus in the insect vector. This is an admirable and suggestive account and one's only criticism is that Fig. 9 is unnecessary or could easily have been reduced and fitted into p. 60. Chapter VII deals with immunity and serological studies, and Chapter VIII with the difficult and controversial problems of the nature of viruses and their classification. Chapter IX discusses the control of virus diseases and the author seems distinctly optimistic of the finding or breeding of resistant varieties. His suggestion, however, that spotted wilt of tomatoes should be made a notifiable disease seems a little surprising in view of its widespread existence in the tomato and its occurrence in so many other horticultural plants. Chapter X is a brief but interesting comparison of plant and animal viruses. The book closes with a bibliography of 105 references "selected as being representative of the main lines of plant virus research" and an index. There are nine text-figures.

The writing of the book shows a certain amount of repetitive phrasing and gives one the impression of having been put together in haste. The book is, however, a good survey within its particular field and should be useful to students.

WILLIAM B. BRIERLEY.

Heredity and the Ascent of Man. By C. C. HURST. Pp. ix+38, with 9 text-figures. Cambridge University Press. 1935. 3*s*. 6*d*.

The author states that "the object of this little book is to provide the general reader with a popular epitome of recent research in genetics in so far as it is concerned with the origin, evolution and ascent of man". The chapters deal with The mystery of life; The laws of heredity; The gene, the origin of life; The gene complex; How evolution progresses; The nature and value of sex; The experimental creation of new species; The ascent of man and mind; The future of man and mind. It is not easy to discuss these subjects briefly in simple language and, here and there, the author makes generalisations and statements with which many workers will not agree. Also there are occasional statements into which it is difficult to read any meaning, *e.g.* "The super-kingdoms of Matter, Life and Mind may be regarded as species writ large in time and value in the course of creative evolution." When Dr Hurst says, "In the light of the gene we no longer see through a glass darkly, and the old-time problems of heredity, variation, sex and species appear as clear as crystal", he will leave ninety-nine out of every hundred biologists envying his vision. The book is a brave attempt but one to be recommended with caution.

WILLIAM B. BRIERLEY.

A Text-book of Mycology. By E. A. Bessey. Pp. xv + 495, with 139 text-figures. Philadelphia: P. Blakiston and Co. 1935. $4.00.

The preface of this volume opens with the statement: "The author's experience, both as a teacher of classes in mycology for twenty-four years, and as a student and teacher of plant pathology, has convinced him that no satisfactory text-book of mycology is available in the English language. Gäumann-Dodge's *Comparative Morphology of Fungi,* while excellent as a reference book and indispensable for advanced students of mycology, is too detailed for use in a first year course in the subject." The excellent smaller text-book by Gwynne-Vaughan and Barnes is apparently unknown to the author. If one compares these three text-books as regards comprehensiveness and detail of treatment Bessey's work falls between the other two. Gwynne-Vaughan and Barnes include about 700 species and genera, Bessey about 1000 and Gäumann and Dodge about 2000, but the quality of Bessey's treatment more resembles that of Gäumann and Dodge than that of Gwynne-Vaughan and Barnes. All teachers will agree that Gäumann and Dodge's book is indispensable for advanced students but many may consider that the present volume is rather too detailed for use in a first-year course in the subject. The book contains nearly 500 tightly packed pages and it would not be easy to omit certain chapters or parts of chapters, as the author suggests, without destroying the symmetry of the work. As it stands many first-year students of mycology will find it a little indigestible, although the writing is so clear that really good students will meet with no difficulty in assimilating it.

The author states that "This book is not a text-book of the physiology of fungi, hence the physiological aspects are subordinated to the morphological, ontogenetical, and systematic features." Not only the physiological but all the more general biological aspects are practically omitted, the only concession made by the author being the inclusion of brief considerations of the genetics of fungal sexuality. A more accurately descriptive title for the volume would have been "Comparative Morphology of Fungi in Relation to Systematics". In many ways it is a tragedy that academic "Mycology" remains so purely morphological and lacking in physiological and biological content. A really "satisfactory text-book of mycology" would surely consider fungi as living things, with vital functioning and environmental relationships: form and structure are only two fragments of mycology. As a description of these fragments however the present book is an admirable statement.

Following a short introductory chapter the author works steadily through the non-filamentous fungi, phycomycetes, ascomycetes, rusts and smuts, basidiomycetes and imperfect fungi. It is all very well done and without going into considerable argumentative detail there is little that one can say about it. One may perhaps welcome the erection of a separate class "Teliosporeae" for the rusts and smuts. Throughout the volume the influence of the work of Gäumann and Dodge is evident, although the author differs considerably in many of his points of view, and in his treatment of the phycomycetes he has obviously been influenced greatly by Fitzpatrick. The imperfect fungi are, for a text-book of mycology, unusually well discussed, some nineteen pages being given to them against four in Gäumann and Dodge's volume and two in that of Gwynne Vaughan and Barnes. The author's treatment of this class is partly based upon the work of H. P. Bender who, in 1931, presented to Yale University a Thesis on the Fungi Imperfecti running to some 2000 pages. It seems a great pity that this work remains unpublished.

As is well known, Prof. Bessey has for long been interested in phylogenetic speculation and, throughout the book, his personal views find frequent expression, although other views are given fair mention, and the author is willing to admit difficulties in some of his own. His morphological attitude leads him to give an impression of specific rigidity and definiteness, reminiscent of the herbarium mentality, which to many fungal geneticists may be just a little alarming. Save in the fungi imperfecti the behaviouristic difficulties in the classification of many fungi are rarely emphasised. Also the author makes occasional statements which may surprise plant pathologists, *e.g.* (p. 219) that "*Sphaerotheca mors-uvae* on the Gooseberry (*Grossularia*) is confined almost exclusively to the berries". Still, as Prof. Bessey remarks (p. 138). "An investigator, with the best will possible, is apt to interpret what he sees in the light of what appears to him to be the most logical series of events" and (p. 182) "Previously held views unconsciously affect the interpretation of the things seen".

Each chapter in the book is followed by an adequate bibliography and, although this is a very minor criticism, on looking through these one feels that perhaps "local" American work is rather over emphasised and English work insufficiently mentioned; the author is obviously familiar with European continental researches. The last chapter, 76 pages, consists of a "Guide to the Literature for the Identification of Fungi" which is based upon a similar guide Prof. Bessey published many years ago in the 21*st Report of the Michigan Academy of Science*, and this, brought up to date in the present book, is a very useful compilation. It is of course already a little behind the times but, as a guide which one can annotate in a personal copy of the text-book, it is valuable. A feature of all the author's citations is that titles and other data are given in full or in easily comprehensible form although, with the *World List of Scientific Periodicals* to hand, this is not so important as it might otherwise have been.

The book is illustrated by 139 excellent figures which, with few exceptions, are either original or taken from recent work and, although one may lament the passing of old friends, one must commend the author on his choice. The book closes with an adequate Index.

For the ordinary botanical student I am not sure that a new and revised edition of Gwynne-Vaughan and Barnes's text-book might not still hold the field as an introduction to the subject but, for students who intend to become plant pathologists and who need a more thorough grounding in mycology than the ordinary botanical student usually receives, Prof. Bessey's volume is strongly to be recommended. It is an admirable mid-way guide to the study of the morphology, sexuality and systematics of the fungi and, as such, it meets a real need.

WILLIAM B. BRIERLEY.

The Arachnida. By T. H. SAVORY. Pp. xi+218; 8 plates. London: Edward Arnold and Co. 1935. 25*s*. net.

Although the Arachnida have received their fair share of attention in natural history books, and indeed have by no means been neglected by zoologists in general, there are very few text-books devoted solely to the class and this new work should fill an evident gap. The subject is treated primarily, although not wholly, from the morphological point of view. Sufficient information is given on the systematic side to enable the student to classify his specimens into families or subfamilies, and useful maps showing the geographical distribution of the different Orders and in some cases families are provided. There are also useful chapters on fossil Arachnida and on the "doubtful" Arachnida such as the Pycnogonida and Tardigrada. The author's treatment of these latter groups and his brief account of the Trilobita is much to be commended since it enables him to refer to the points in which they show Arachnid affinities, without confusing the reader or admitting them as members of the class.

A peculiar but nevertheless not unwelcome feature of the book is the addition to most of the chapters of a short essay, or essay in summary, which the author designates as an "excursus". Various subjects more or less related to the study of the Arachnida are dealt with in this manner, and the reader will be able to remind himself of zoological topics ranging from Gaskell's ingenious hypothesis of the origin of the vertebrates to the odd story of the mythical Gibbocellum. A chapter is provided on "economic arachnology" which, although brief, will at least give the reader who is not an applied biologist some idea of the economic importance of the class. The book also contains good indices and a useful list of references, not only to original works but also to works in which more extensive bibliographies may be found. The illustrations are good and the general make-up and printing excellent.

As a whole, the work will perhaps commend itself most readily to the student or post graduate who is working for a higher degree in zoology and no less to his supervisor. To the applied biologist the value of the work, apart from its purely scientific interest, will probably be found in the fact that it will enable him profitably to remind himself of much that he once knew (or at all events was once taught) but has now forgotten: and it is surprising how often those whose practice is most closely confined to applied zoology nevertheless meet with questions involving the more academic aspects of their subject. In only one respect must the reviewer presume to differ from the views set forth in the book, and this curiously enough arises from the opening sentences of the preface. The author remarks that his purpose in writing was to attempt to give to arachnology something of the unity and status of the individual sciences possessed by entomology, and he concludes by saying that there "has long been a natural dominance of entomology over most other branches of zoology". To an entomologist brought up in the old traditions of zoology, the division of the major subject into a number of separate and often disconnected units, each with a distinct and formidable terminology understood only by the specialist, is rather disquieting. Is there any reason why entomologists should not study the Arachnida? Applied entomologists cannot avoid doing so, and probably more field work on the Arachnida is carried out by "entomologists" than by any other group of zoologists. Also, is it true that entomology (restricting the term to the Insecta) has long dominated other branches of zoology? In applied zoology this may be the case, but, at least until quite recently, the Insecta were deplorably neglected in the average university course of zoology, and even the Arachnida came in for better treatment. The fact, however, that little sympathy is felt for the author's views on these particular questions in no way detracts from the real value of the book, and these few remarks may be treated as an "excursus". Most reviews, like scorpions, have stings in their tails!

J. C. F. FRYER.

Garden Science. By JOHN GRAINGER. Pp. 265, 110 figures. London: University of London Press. 1935. 4*s*. 6*d*.

Among the thousands of gardeners, professional and amateur, in this country there must be hundreds who have had no scientific training but who would like to know something of the principles of plant behaviour, and the reasons for the methods used in gardening practice. They will find in this book a readable and concise account which will give them a new interest in the plants with which they deal and a better understanding of the theory underlying the art of horticulture. The author's association with the teaching profession has led him to cast the book into a form suitable for use by teachers of elementary science in schools, and suggestions are made for experimental demonstrations, some old, some commendably original, suited to the young idea.

The first four chapters comprise an excellent brief account of the physiological processes of plants, with illustrations drawn from horticultural practice. As would be expected from one associated with Prof. Priestley's school, much consideration is given to the physiology of vegetative propagation. This is all to the good, for information on this subject has so far been available only to those with access to the origina literature. In such small space as these few chapters it is impossible to avoid some errors in clarity, but if an amateur reading p. 140 attempted to prune a *Lonicera* and a *Philadelphus* in a similar manner to a *Hydrangea hortensis* he would undoubtedly be very disappointed with the results in the following season.

Chapters VI, VII and VIII, comprising nearly a third of the whole book, deal with the diseases and pests of plants. There are so many books, large and small, available on these subjects and so few on physiology as applied to horticulture, that one would almost have preferred to see this left out and the main part expanded. Nevertheless the account of diseases and pests is succinct and useful, and will form a guide to procedure for those in trouble with attacked plants.

An appendix dealing with experimental method will doubtless be revised in future editions; the advice given is good so far as it goes, but is altogether too brief to be of much use, and in any case the modern replicated experiment such as the Latin Square layout is hardly suitable for the school garden. Further, a Latin Square experiment in which the sums of the separate rows and columns were all equal would be a truly astonishing occurrence. On such uniform soil replication would serve no useful purpose.

These criticisms are, however, small compared with the undoubted interest and value of the book to the horticulturist and to the first-year student.

R. H. STOUGHTON.

Plant Physiology. By MEIRION THOMAS. Pp. xii+494, 57 figures. London: J. and A. Churchill, Ltd. 1935. 15*s.*

A new book on plant physiology is always sure of a welcome. Even if it contains little that is new or expounds no new and original theory the methods of approach, or the bringing together of known facts in a different conjunction, can be stimulating, or even provide in an indirect manner new clues to workers in specialised fields. It is true that text-books are not written for the research worker primarily, but the wise specialist will take note of the new text-books, for it is only by the aid of these condensed forms that he can manage to keep abreast of developments outside his own particular field.

The author of the present volume has no special axe to grind; he is at pains only to present in a logical and clear manner the basic principles of the functional processes in plants, to outline the theories of other workers in explanation of these principles and to suggest the nature of some of the outstanding problems which still confront the plant physiologist. The approach to the subject is along the lines of the classical physiology, the intimate study of the biochemical and bio-physical mechanisms involved in the individual functions. The integration of these functions which results in the growth and development of the plant receives little attention, and the physiology of reproduction is not touched upon. As a consequence the whole field of the influence of the environment upon plant behaviour is left out of consideration except in so far as the components of the environment affect the separate processes.

The book is therefore a text-book in the most restricted sense of the word and its use by students is open to the danger of presenting too narrow a view-point to the minds of those who have not yet developed the critical faculty and the power to "see a subject and see it whole". In this respect it belongs to the class of the older school of morphological and taxonomic botany. This is not meant as a criticism of the book; the author's avowed purpose and his recognition of the omissions are clearly set out in the preface. Within the limits which he sets himself the volume will be a valuable reference and class book.

The book is divided into four parts. Part I deals with the physical and chemical activities of protoplasm and the nature of enzyme action. Part II is concerned with absorption, translocation and transpiration. Critical consideration is given to the work of Mason and Maskell on translocation of solutes, but little attention is paid to the researches of Loomis or Curtis on the upward movements of solutes in the phloem and the work of Birch-Hirschfeld is not mentioned. Nutrition and metabolism are dealt with in Part III and, as would be expected from the author's own specialisation, the chapter on respiration is particularly well done. Part IV, on growth and movement, has obviously presented the greatest difficulty in deciding what to include and what to omit. Recent work on plant hormones is treated clearly and succinctly, but the possibly related subject of dormancy of seeds and buds might with advantage have been expanded. The book concludes with two appendices on the chemistry of metabolic processes and notes on physical chemistry respectively, and the separation of these parts from the general text will be found useful by the student.

A well-selected bibliography of 163 citations and an author index add to the value of the book to advanced students.

It is to be hoped that in future editions it will be found possible to include the matter dealt with in footnotes in the main text. Footnotes occur on more than half the total number of pages and constitute a continual irritation in reading.

R. H. STOUGHTON.

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SOME APPLIED BIOLOGICAL ASPECTS OF PROBLEMS RELATING TO PLANT-PARASITIC NEMATODES

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ADDRESS OF THE RETIRING PRESIDENT OF THE ASSOCIATION OF APPLIED BIOLOGISTS, DELIVERED AT THE ANNUAL GENERAL MEETING ON FEBRUARY 14, 1936

LADIES AND GENTLEMEN,

The occasion of a presidential address provides an opportunity for surveying one's scientific interests and of attempting some kind of appraisement of the significance of one's subject in the large field of applied biology. I propose, therefore, to speak to you this afternoon on certain topics connected with plant-parasitic and related nematodes. As this branch of biology is a somewhat specialised and restricted one, even in that wide realm of applied biology embraced by parasitology, and as there are but comparatively few workers engaged in it throughout the world, I want at the outset to attempt to define its position. This is perhaps the more desirable since it would probably not be very wide of the mark to hazard a guess that among my audience there are a number who have but the merest passing acquaintance with the lowly animals called eelworms. Having illustrated the scientific background of the subject, mainly in relation to human and veterinary helminthology, I propose to say something on its history and growth and then to turn to the consideration of a few of its applied biological aspects in problems of agriculture and horticulture. Finally I shall draw your attention to the almost total lack of facilities for instruction in the subject at any of our centres of higher education.

RELATION TO HUMAN AND VETERINARY HELMINTHOLOGY

Any attempt to separate the free-living and plant-parasitic nematodes from the general body of the Nematoda so as to constitute them a distinct phylum, as was made by the late Dr N. A. Cobb in his paper, "The orders and classes of Nemas" (1919), is, I think, to be deprecated.

A nematode is a nematode whether it lives free in soil or water or parasitic in animal or plant. For this reason I want to say something on the relation of the subject to that wider field of parasitology which embraces the study of the worm parasites of man and animals. Human and veterinary helminthology are old sciences covering a much greater range of parasites than those met with in the study of eelworms. They include within their purview the Platyhelminths or flatworms, which comprise the Trematoda or flukes and the Cestoda or tapeworms as well as the Nematoda or roundworms. We are concerned only with the Nematoda which, as parasites of man and animals, includes at the present time a pretty formidable array of genera and species which, though exhibiting great diversity of shape and size are yet readily recognisable as possessing certain common structural features. This enables us to group them in a fairly compact and self-contained class with certain distinctive characteristics. It is, in fact, this very uniformity of structure and anatomy amongst the Nematoda that renders it difficult to find any grounds of support for the attempt to separate the free-living and related forms from those parasitic in animals.

Let me say a few words on the antiquity of knowledge of the worm parasites of human beings. According to Faust's book, *Human Helminthology*, it is in the Ebers Papyrus of the sixteenth century B.C. that the earliest record of a helminth considered as a pathogenic organism is to be found. It is impossible to say whether the worm in question was a tapeworm or a roundworm, but symptoms were attributed to its presence in the bowels and a remedy advised for its expulsion. The Chinese in ancient times were acquainted with the use of certain vermifugal remedies. Moses, who doubtless obtained his knowledge from Egyptian priests, laid down laws of sanitation and hygiene to be observed by the Hebrews. In all probability the fiery serpent which attacked the Israelites in the wilderness of Sinai was none other than the guinea worm, *Füllebornius* (*Dracunculus*) *medinensis*, sometimes called the dragon worm, the long female of which gives rise to a swelling on the foot, ankle or leg which, on rupturing in water, sets free the larvae of the parasite. We may perhaps recognise in the method employed even to-day of extracting the parent worm by winding it out a bit at a time on to a stick a likeness to Moses's winding the serpent round a rod. It is also reasonably suggested that the separation of animals into "clean" and "unclean" as laid down in the early books of the Old Testament was on the basis of those free from or infected with parasites. Coming to later ages, Faust also tells us that the oldest record of the Christian era of interest to helminthologists is that of

Avicenna, an Arab physician who lived between A.D. 981 and 1037. He described four kinds of worm parasites of man, three of which were nematodes. In each case symptoms are given and vermifugal remedies prescribed, such as santonin and extract of male fern, which are in use at the present day.

Further progress in our knowledge of the nematode parasites of man and of domestic animals had to wait till post-Linnean times. Linnaeus listed four nematodes from humans in his *Systema Naturae* (1758–67), but gradually other parasites of man and animals were recognised though the working out of their life histories is mainly the result of researches conducted in much more recent times.

I turn for a moment or two to illustrate the relation of free-living nematodes to those parasitic in man and animals to re-enforce my point that the subject should be considered as a whole. There are two nematode parasites of man, namely, *Strongyloides stercoralis* and *Ancylostoma duodenale*, both of which pass through developmental stages outside the host in faecal matter. The eggs of *Strongyloides stercoralis* give rise to larvae which, under certain circumstances, develop into small eelworms consisting of male and female forms. After copulation the females lay eggs which produce larvae and these, instead of repeating the sexual generation, remain as slender, filariform larvae capable of infecting through the skin. The free-living sexual males and females can easily be confused with saprophagous free-living eelworms and in fact were at first classed as belonging to an old genus, *Anguillula*, which has served as a sort of *omnium gatherum* for many forms whose affinities have been obscure. Looss (1911, pp. 216–19 and 365–6) paid close attention to this matter of the possible confusion of free-living nematodes with developmental stages of a parasitic form in his researches on the human hookworm, *Ancylostoma duodenale*. This parasite lives in the small intestine and produces eggs which pass out in faeces where, on hatching, they give rise to what are termed rhabditiform larvae, so called because the mouth cavity and the muscular oesophagus are similar in appearance and structure to the corresponding regions of members of the genus *Rhabditis*, one of the commonest and most widespread of free-living nematodes. In fact these rhabditiform larvae may easily be confused with young forms of many *Rhabditis* species occurring in soil.

I may perhaps venture on one further example in illustration of the need for a comprehensive view of the nematodes as a whole group. In the United States of America shortly after the end of the Great War a paper was published describing the occurrence in the faeces of a good number of American soldiers of the eggs of a new nematode parasite which was provisionally named *Oxyuris incognita.* No particular clinical symptoms could be associated with the occurrence of such eggs which, by the way, did not persist for long in the faeces of any of the soldiers, and, in spite of the administration of various vermifuges to the long-suffering patients, there was complete failure to dislodge the hypothetical parent worms which were supposedly producing the eggs.

The matter was very puzzling until it was shown in 1923 that the eggs were identical in appearance and size with those of the plant-parasitic eelworm known as the root-knot nematode, *Heterodera marioni.* The soldiers had, in fact, merely been eating potatoes or some other vegetable infested with this parasite whose eggs, during the process of digestion, had become mixed with the contents of the intestine and had finally been voided. The moral of this illustration scarcely needs pointing, but it does show that if there is need in the study of nematodes for viewing the group whole rather than in a piecemeal manner, that need applies equally to the medical and veterinary parasitologist in the difficult business of identifying parasites from eggs.

PLANT PARASITIC NEMATODES: HISTORICAL

I now pass to a consideration of the growth in knowledge of eelworms, particularly of plant-parasitic and free-living forms. Probably the first eelworm with which early naturalists, and especially early microscopists, became acquainted was the vinegar eelworm, now called *Turbatrix aceti.* This organism, which is often found in vinegar in many parts of the world, was mentioned as early as 1656 by Petrus Borellus. Later on it was studied by Leeuwenhoek who, as Dobell has shown in his fascinating book, *Leeuwenhoek and his Little Animals*, was the first to discover that the eggs develop within the female which becomes viviparous and to show that the globules in the intestinal wall are composed of fat. These facts were set forth in a communication to the Royal Society of London in 1676 and make extremely interesting reading. It was not until nearly another century had elapsed that further knowledge of eelworms began to grow as books on the microscope and the wonders it revealed made their appearance. Baker in 1743 wrote on the sour paste eelworm, whilst a number of other early writers, including Adams (1771) and Hooke (1780), discoursed at length upon it and on how to grow it. This organism soon became confused with the vinegar eel, and I may perhaps mention in passing that it was not until as late as 1922 that it fell to my lot to settle the question of their separate identity.

It was Needham, who, in 1743, whilst studying grains of smutted wheat, discovered the galls or cockles caused by the eelworm now called *Anguillulina tritici*, and in so doing took the first step in bringing to light knowledge of plant-parasitic nematodes. Information on the habitat and behaviour of eelworms came from a variety of sources, and we are indebted to the work of many cecidologists for our knowledge of plant galls due to nematodes. It was in 1799 that Steinbuch gave an account of the galls in the flowers of certain grasses caused by eelworms which he called *Vibrio agrostis* and which we now recognise as belonging to the same genus as the wheat eelworm. Another species giving rise to galls on the leaves of Fescue grasses was described by Hardy in 1850 as *V. graminis*. Plant galls are obvious malformations, and it is easily understandable that they should receive attention from naturalists and others and that the contained organisms should be described especially if the plant happened to be of economic importance. So it came about that swollen and galled shoots and roots and various other malformed structures were examined. Berkeley in 1855 found the larvae of the root-knot eelworm, now known as *Heterodera marioni*, in the swollen roots of cucumbers grown in an English greenhouse. In 1859 Schacht found the little cyst-like females of another species of eelworm attached to the rootlets of sickly sugar-beet. This species was later given the name of *H. schachtii* by Schmidt in 1871, and from that time onwards there grew an immense literature in connection with sugar-beet sickness and the intricate and difficult problem of its control; a problem which to-day is far from completely solved. Kühn, in 1858, published an account of a diseased condition in the flower-heads of teasel or fuller's thistle and called the eelworm associated with it *Anguillula dipsaci*. This was recognised a few years later by Bastian as a species of his genus *Tylenchus*, but it had already in 1859 been placed by Gervais and van Beneden in their genus *Anguillulina*; a fact which was lost sight of for very many years with the result that the parasite has generally been called *Tylenchus dipsaci* or *T. devastatrix*. It is only in the last few years that, in attempting to apply the rules of zoological nomenclature impartially, we have come to see the error of our ways and now call it by its proper but tongue-twisting name, *Anguillulina dipsaci*.

I must say a little more on the early history of this parasite because of its widespread attacks on agricultural and horticultural crops. In 1825 Schwertz in Germany had noted the occurrence of a stunting disease attacking rye, clover, oats and buckwheat and had given the name "stock" to the condition found in rye. About 1867, Kamrodt found eelworms in rye showing the "stock" symptoms, and in 1869 Kühn showed that these eelworms were the cause of the disease. He also proved that the worms causing disease in teasel heads could attack rye and give rise to typical "stock" symptoms. In the course of the next 10 or 15 years the parasite was found in lucerne, hyacinths and onions, and this led to the important researches of Ritzema Bos (1888–92), which brought to light the fact that one and the same organism was responsible for disease in all cases.

In order to complete this brief survey of the progress in knowledge of plant-parasitic eelworms I must give some account of certain root-infesting species of *Anguillulina* which do not provoke gall formation but give rise to necrosis of the tissues, and a few words also about members of the genus *Aphelenchoides* which are so important as parasites of horticultural crops. The eelworm now known as *Anguillulina similis* is an important parasite of certain tropical and subtropical plants of economic importance such as coffee, tea, pineapple, sugar-cane and banana. It was first described by Cobb in 1893 from soil about the roots of banana in Fiji. In 1898 it was again found by Zimmermann in coffee roots and given another name. Cobb described it again under still another name in 1909 from Hawaiian sugar-cane roots, and still later in 1915 straightened the matter out when he found adults of both sexes in roots of Jamaican bananas when he recognised it as the same species he had originally found in Fijian soil. A somewhat similar history attaches to another root parasite, *A. pratensis*, which incidentally illustrates the importance of faunistic studies of soil nematodes. This eelworm was first described by de Man in 1881 from soil in Holland as a free-living species. Zimmermann in 1898 found it along with *A. similis* in coffee roots and called it *Tylenchus coffeae*. Cobb described it afresh from cotton and violet roots under the name of *T. penetrans*, whilst in 1924 Rensch described it as a pest of cereals in Germany under the name of *Aphelenchus neglectus*. Finally, in 1927, Steiner cleared the matter up by showing that the parasite was one and the same as de Man's *Tylenchus pratensis*.

All the species of *Anguillulina* so far mentioned are internal parasites, but there is one interesting member of the genus which is ectoparasitic on rice in the Ganges delta, causing a disease mainly of the inflorescence called "Ufra". Our knowledge of this is based almost entirely on the work carried out on it by a past president of this Association, Dr E. J. Butler, published in two papers which came out in 1913 and 1919.

The strawberry eelworm, *Aphelenchoides fragariae*, was first described by Ritzema Bos in 1891 from strawberry plants suffering from "cauliflower" disease in England which were sent to him by Miss Ormerod. In

1893 he described another species, now known as *A. olesistus*, which gives rise to leaf blotch in ferns and is also destructive to begonia leaves and other ornamental plants. In 1911 Schwartz published a more detailed study of these forms and separated from them the species which is such a serious pest of chrysanthemum leaves under the name of *A. ritzema-bosi*. Since then and in quite recent years much has been published on this destructive pest and many methods for its control have been tested. Lastly, I may mention the eelworm, *A. cocophilus*, which is associated as a systemic infection in coconut palms suffering from the deadly "red ring" disease in the West Indies. Knowledge of this dates from the years 1919 and 1921, when the important papers of Nowell and Cobb dealing with it were published.

I have briefly sketched something of the growth in knowledge of some of the more important plant-parasitic eelworms. Investigation of these organisms is still in progress, on forms which have been imperfectly or inadequately described in older works and on recently recognised forms, with the result that in recent years much new information has been brought to light. Re-study of forms described years ago has led to their better description and differentiation, standards of accuracy and refinements in morphological studies and technical description have been set up to the betterment of the science in all directions. In addition, we are beginning to know something about the pathology of the various disease conditions. This line of enquiry, which has been rather neglected in the past, now promises to throw light on the nature of the offensive maintained by the parasite and on the defences set up by the host plant.

Concurrently with increasing knowledge of plant-parasitic species there has been steady progress in knowledge of those forms which are found in fresh and salt water, free-living in the soil and in all sorts of decomposing organic media. Some of these we now know to be carnivorous predatory creatures which feed on other minute animals in the soil, including parasitic and saprophagous eelworms. Many important faunistic papers dealing with soil nematodes have appeared from time to time, and this type of work still goes on. It is of considerable importance to the investigator of plant-parasitic and related forms if only for the reason that two of the genera attacking plants and insects also contain numerous species which are free-living. In addition, one often finds numbers of eelworms in diseased plant tissues, and it is essential for us to possess a pretty wide knowledge of the forms encountered so as to be in a position to distinguish them from the disease-producing species. For reasons such as these it is desirable that a wide view of the whole subject should be maintained by the worker on these organisms.

SOME PRESENT-DAY PROBLEMS OF PLANT HELMINTHS

Specialised technical questions of nematode structure and systematics would scarcely be of interest to the members of this association, but I hope that some of the problems connected with plant-parasitic species of practical importance in agriculture and horticulture may not be inappropriate for discussion in an address of this character.

Gall formation. The species of eelworm which give rise to galls in the flowers and on the leaves of cereals and grasses are not of great economic importance at the present time. The methods of combating and controlling "cockle" in wheat are simple and easily applied. It is easy enough to omit wheat or a susceptible cereal for a season or two and not difficult to obtain a clean supply of seed for sowing. The grass galls do not cause serious damage to pastures and leys, at any rate in this country. From the scientific standpoint, however, gall formation is of importance if we wish to have any clear ideas on the pathogenic behaviour of the parasites concerned. For example, it has been widely assumed, because all the plant-parasitic nematodes possess a spear-like structure in the head, that this organ is the weapon which inflicts damage to the host tissues. Such a simple explanation is, I believe, very far from the truth, as may be illustrated from many cases of plant galls and in particular from those caused by a species of *Anguillulina* giving rise to galls on the leaves of an Indian pasture grass, *Andropogon pertusus*, which I studied a year or so ago (Goodey, 1934). The galls are small, round, shot-like swellings, arising sharply from the leaf-stem of leaf-sheath, and sections through them show that marked changes in the tissues occur as a result of their invasion by the parasite. The cells of the upper and lower epidermis become enlarged, and the mesophyll cells undergo remarkable hyperplasy and hypertrophy resulting in the formation of a mass of tissue composing the gall. A cavity forms in the centre of the mass, and in this the parasites mature and reproduce. Examination of the cells composing the gall show that they undergo profound modification. Those lining the gall cavity are large, have finely granular, vacuolate protoplasm, and each contains a large nucleus with two or more nucleoli. These cells are no doubt to be regarded as nutrient in function; probably exosmosing food substances on which the parasites feed. Outside the nutrient zone we find enlarged cells whose walls have become considerably thickened by the deposition of reticulate or scalariform thickenings.

I do not know of any other plant galls resulting from nematode attack so highly organised structurally as those caused by this species. In fact for comparable structures we must turn to galls resulting from the attack of certain cynipid insects in which also we find an inner nutrient zone of cells surrounding the gall cavity and outside this a layer of cells whose walls are considerably thickened.

How does such a gall arise? Surely not as a result of the action of the spear-like mouth organ, since many of the cells exhibiting modification are not accessible to the parasite itself, but probably in much the same way as do galls caused by cynipid insects. Kostoff and Kendall (1929 and 1930), to whose work on galls I am indebted for any ideas I have on the subject, have suggested that a gall arises as a reaction on the part of the plant to the presence of irritant substances poured out by the parasite. Such substances stimulate the plant tissues to increased metabolism which is expressed in more rapid growth and greater cell division. In the immediate vicinity of the source of the irritants their action is so strong that the cells do not continue to divide but remain large and so constitute the nutrient zone. Some, in fact, break down under cytolytic action and so form the gall cavity. Outside the nutrient zone the irritant substances act under optimal conditions and bring about hypertrophy and hyperplasy which results in the mass of cells comprising the gall. Beyond this region there is apparently limitation of their action and gall formation ceases.

In immunological terms the plant can be regarded as capable of elaborating antibodies or protective substances in response to the presence of antigens in the irritant substances poured out by the pathogen. The plant's protective mechanism is brought into play and a time comes when the antibodies are present in sufficient quantity to neutralise the stimulant effects of the irritant substances and gall formation is brought to an end. It is in the zone of neutralisation that we find the plant reacting by the thickening of its walls in both cynipid galls in a nematode gall of the complex type I have just described.

In other nematode galls so far investigated anatomically we do not find so elaborate a structure as that caused by *Anguillulina cecidoplastes* on *Andropogon pertusus*, but it is none the less probable that somewhat similar factors are at work in their formation. Much remains to be done in this line of investigation, and a very varied and interesting field awaits the attention of some future worker. In root galls caused by *Heterodera marioni* and *H. schachtii* giant cells are produced just outside the vascular cylinder which probably serve as nectaries or special food reservoirs from which food substances are exosmosed and on which the parasites feed.

PROBLEMS CONNECTED WITH *ANGUILLULINA DIPSACI*

I propose now to deal briefly with one or two matters connected with the stem or bulb eelworm, *Anguillulina dipsaci*. First with a disease of bulbous irises caused by this parasite, and then with certain questions relating to "tulip root" in oats and stem sickness in clover. I bring up the eelworm disease of iris bulbs for consideration partly because of its growing importance and increasing incidence but also because it apparently presents us with a case of a disease arising within the past ten years or so. The earliest record of eelworm attack on iris bulbs is that of van Poeteren in 1925, who stated that a large part of a stock had gone rotten probably because they had been packed in boxes too long. His observations were made in 1924, so it is quite probable that attack on iris bulbs had been proceeding for some time; how long it is impossible to say, before it became thus noticeable. No further records appeared until 1929 when I listed attack on Spanish iris bulbs, but it is interesting to note that the observations recorded in 1929 were made in November 1924 on material sent to the Ministry of Agriculture Plant Pathology Laboratory, and the parasites were found by the staff there and by myself. The origin of these bulbs I do not know. In 1932 Tschaen reported attack in *Iris filifolia* and *I. tingitana* from the south of France. In the next year Steiner and Buhrer (1933) brought out a short paper and described symptoms of attack in Spanish iris (*I. xiphium*), Dutch iris (*I. xiphium hybridum*), English iris (*I. xiphioides*) and Tangerian iris (*I. tingitana*). They recorded having found the parasite in bulbs imported from Holland and from a planting in Virginia.

In our own country Hodson (1934) has given an account of the symptoms of attack in the resting bulb and under growing conditions in the field. Steiner and Buhrer's and Hodson's work shows clearly that evidence of attack is only apparent on the removal of the outer scaly bulb tunic, when more or less extensive diseased, discoloured areas are found towards the base and on the side of the fleshy bulb. When affected bulbs are cut transversely the diseased areas are found to penetrate more or less deeply into the tissues of the bulb, and in these areas the parasites are found in good numbers. Attack would therefore seem to be direct from the outside inwards and not downwards into the bulb rings as in narcissus. Diseased bulbs when planted either rot completely or produce poor discoloured leaves and are flowerless. They may succeed in producing daughter bulbs which in turn become attacked. It is interesting to note that the foliage has never been found to show spickles or blister-like lesions so characteristic of attack on narcissus and hyacinth leaves. So far attempts by Steiner and Buhrer and by Hodson to control by the hot-water treatment have proved ineffectual. Hodson submitted infected bulbs for 1 and 2 hours to water at 110° F. in December, but though treatment for 1 hour was sufficient to kill the parasite all the bulbs had died by the following March. On the other hand Staniland (1935) has found that bulbs of Iris "Imperator" treated for 50 min. at 110° F. in early autumn successfully withstood the hot-water treatment. Newton in British Columbia has also had failure with hot-water treatment in the case of infected irises. He reports in a letter that a recent consignment of Dutch iris bulbs imported from England showed a 100 per cent. infection, and though hot-water treatment was a failure some success at control has been obtained by soaking the bulbs for 3 hours in silver nitrate-potassium cyanide solution, *i.e.* by the process worked out at the Saanichton Laboratory and recommended for the treatment *in vacuo* of infected narcissus bulbs (*vide* Newton, Hastings and Bosher, 1933). As to whether this strain of the parasite is the same as that attacking narcissus, the evidence of experiments carried out by Steiner and Buhrer and by Hodson point to the two races being distinct, since reciprocal infections were not established. Thus, although this disease of irises has apparently arisen in quite recent times, we are not, I am afraid, in a position to point to its exact origin. Bulbous irises are frequently used to follow plantings of narcissus, but if the narcissus strain of the parasite originally transferred to iris it has already become so well acclimatised to its new host that it will not now infect narcissus.

Turning to "tulip root" in oats and stem sickness in clover it may be noted that both conditions are fairly widespread in this country, as is shown by the records appearing from time to time in the *Monthly Summaries of Plant Pests and Diseases* issued by the Ministry of Agriculture. One of the most difficult things to account for in the case of both diseases is their sudden appearance in fields where the preceding crops in a rotation are non-susceptible ones or, if susceptible, have shown no obvious evidence of eelworm infestation. Two possible explanations suggest themselves, (1) that the parasite is seed-borne or (2) that there may be a light natural infection on weeds from which the organism passes over to the crop. As to the first possibility, Robertson (1928) showed that *Anguillulina dipsaci* may occur in the panicles of oats (though not actually within the seed coats) which have been harvested from an affected crop. Hodson (1933), too, has recorded the occurrence "tulip root" at different centres, the seed sown having been obtained from a common source which was a diseased crop. It is known, too, from Cobb's investigations, that red clover and lucerne seed, even in the cleaned state, can carry the parasite in the dried viable condition. It is known also that the parasite can be dispersed within the seed coat of the weed *Hypochoeris radicata* (cat's ear), a fairly common composite, both in the United States and this country. The potentialities of the parasite being seed-borne require still further investigation, especially in connection with the sporadic incidence of disease. On the question of it being harboured by various weeds and of these possibly serving as reservoir hosts much remains to be done, but we know from Quanjer's (1927) researches in Holland that certain weeds served as the source from which the parasite spread and attacked potatoes in his experiments. However, this proved to be a polyphagous race, and the situation is generally complicated by the fact that most of the races of the parasite attacking agricultural crops are highly specialised biological ones which restrict their attacks to a particular host or to a narrow range of hosts. A considerable number of weeds have been recorded as hosts of the parasite, and on two occasions I have myself found one of the common buttercups showing infestation under experimental conditions where the source of infection was the red clover strain of the parasite. Observations of this character are of importance, as they suggest a possible answer to the problem of the occurrence of disease when the particular crop has been absent for some years. If the parasite has been maintaining itself as a light infestation on a fairly common weed we may the more easily account for its apparently sudden appearance on the susceptible crop when next it comes in the rotation. Some such explanation seems to be required, since all the evidence available points to *Anguillulina dipsaci* being an obligate parasite, and it is extremely difficult to suggest an explanation of its surviving in soil under normal moist conditions in the absence of some host or other for more than 12 or 18 months. We know, of course, that the pre-adult infective larvae can survive in the dry condition for periods up to 6½ years and revive on moistening, but this is a very different matter from life in moist soil where we must envisage them as moving about and slowly using up their reserve stores of fat. More information is still needed on the biology of the parasite under normal soil conditions. Robertson (1935) has shown, in the case of samples of soil from an oat field in which the crop was severely attacked in 1926, that in the following year a heavy infestation was set up in oats sown in pots. In 1928, 30 per cent. and in 1929, 3 per cent. of the oat seedlings grown in pots of soil taken from the field showed infection. The field itself during these years was down to grass. By 1930 the soil was no longer infective to oats, and the parasite had apparently died out. It had, however, persisted for 3 years in the absence of the appropriate host, and this is a much longer time than one would have expected it to be able to do. It will be evident from what I have said that the problem of the complete eradication of the parasite is an extremely difficult one. Measures to ensure a supply of clean, parasite-free seed and weed destruction immediately suggest themselves, but the latter is by no means easy to accomplish. Sowing down to grass can be recommended as can well-devised rotations, but in the case of "tulip root" in oats, it would be advisable to omit field beans, since there are at least two cases on record of beans being attacked following disease in oats. Another approach to control in the case of "tulip root" which is now receiving attention is the use of disease-resistant varieties, but the time is not yet ripe for much to be said about this beyond an indication that there is evidence that certain varieties show a marked resistance to attack when grown under conditions which lead to severe injury in other varieties.

Hot-water treatment. Discussion of problems connected with *Anguillulina dipsaci* affords a convenient opportunity to say something about hot-water treatment and some of the present-day work on it, since it is in relation to narcissus bulbs infested with this parasite that so much work has been and is being done. The subject as a whole is too big to be dealt with completely in an address of this kind, but it may be noted that there are three main lines along which the use of heat either as steam or hot water has been directed in attempts to control eelworm diseases (I am leaving out any consideration of the so-called vapour heat treatment), (1) treatment of soil, (2) treatment of the growing plant, (3) treatment of the resting plant. It is convenient to separate (2) and (3) though they are intimately connected.

(1) The use of steam for the treatment of greenhouse soil was practised in the United States as long ago as 1894, and the method was successfully elaborated by Stone and Smith in their paper on the root-knot eelworm published in 1898. In our own country a considerable amount of work was done on the steam treatment of soil in connection with investigations on the partial sterilisation of soil at Rothamsted and elsewhere round about 1908–13. This work links up with the American work, although it was not primarily directed to the killing of parasitic eelworms but to the wider problem of soil sickness. In the work of

Bewley on soil sterilisation the practical aspects of the application of heat to soil have been placed on a sound and firm foundation.

(2) The successful treatment of the living plant infected with eelworms as a method of control was reported by Marcinowski in 1909 in the case of both ferns and begonias, the leaves of which were infected with *Aphelenchoides olesistus*. She immersed plants in water at 50° C. for 5 min., thereby killing the parasites but leaving the plants uninjured or but temporarily retarded. The more extensive application of such a method of treatment has had to wait for much more recent years, but we now know of its successful use with strawberry runners and violet plants treated for 30 min. in water at 110° F., followed by plunging in cold water. In the case of chrysanthemums the stools can be given a similar treatment of 30 min. in water at 110° F., before the cuttings are taken; the young growths being free from infection. The treatment retards the sprouting of the stools for 2 or 3 weeks, so it is advisable to administer it 2 or 3 weeks earlier than the normal time for taking cuttings. Lily of the valley crowns are reported by Thompson (1934) as being freed from attacks of *Anguillulina pratensis* by treatment with water at 45° C. for 30 min. Hodson (1935) has reported that phlox plants attacked by *A. dipsaci* and treated during the resting stage have responded well to treatment in hot water at 110° F. for 30 min., the new shoots being free from infection. The method, in fact, bids fair to becoming one of the most useful and efficacious for the control of eelworm infestations in plants which are of a size suitable for the hot-water bath.

(3) The treatment of narcissus bulbs by the hot-water method to rid them of *Anguillulina dipsaci* had an origin independent of the two already mentioned, in that it was taken up primarily in attempts to free them from the attacks of narcissus bulb flies. In 1902 it was suggested by Saunders, in a note written by Wilks, that steeping fly-infested bulbs in water at 115° F. for 20 min. should be tried. Theobald reported in 1911 that good results could be obtained by immersing bulbs in water at 120° F. for about 10 min.; I am told, however, that the bulbs were killed. To Fryer, however, belongs the credit for the most successful of these early attempts to control these flies. In 1915 he gave an account of work in which infested bulbs were treated for 1 hour in water maintained at 110° F., by which means the fly larvae were completely destroyed, and it was incidentally established that the bulb eelworm, if present, was controlled with a 90 per cent. efficiency. Fryer's preliminary investigations in reality formed the basis for the later work of Ramsbottom, whose researches led to the standard treatment of bulbs for 3 hours in water kept at 110° F.

Fryer's share in thus establishing a good firm foundation on which so much later work has been erected has been almost entirely overlooked, I have thought this a not inappropriate occasion on which a long overdue estimate of the place and significance of Fryer's work might be made.

A great deal has been written on the standard treatment since Ramsbottom's papers appeared in 1918, and the method has been very widely adopted, with the result that probably hundreds of tons of bulbs are treated annually. Nevertheless, complete eradication of the narcissus race of the parasite has not been accomplished, and in the past 2 or 3 years a good deal of research has been done on various aspects of the problems involved in the process.

Staniland brought out a paper in 1933 in which he dwelt mainly on two aspects: (i) the time taken to kill the eelworms, and (ii) the time required to bring the centre of bulbs of various sizes to 110° F. Having determined that it takes approximately 20 min. to kill specimens of the parasite taken from leaf lesions in water at 110° F., he then showed that the time required for the centre of a bulb to reach 110° F. varied according to the diameter and size of the bulb, and that for smaller bulbs a much shorter time was required to effect destruction of the parasite than the standard 3-hour treatment. Length of treatment could, in fact, be varied according to size of bulb, and he claimed that as a result of the shorter treatment several of the adverse effects of the 3-hour treatment, such as flower blindness and splitting of the trumpet, could be avoided.

As was perhaps to be expected, Staniland's results have received a somewhat mixed reception, and it is maintained by some that it is dangerous to recommend a treatment shorter than the standard 3 hours. There can be no doubt, however, that it has served a useful purpose in concentrating attention on various details of the general problem. For example, if bulbs are left rather late before treating, say till October, ample time is given for the accumulation at the base of bulbs of masses or tufts of the so-called eelworm wool which consists of enormous numbers of pre-adult infective larvae. As these become coiled and dry they are very resistant to desiccation, and it is clear from the results of experiments carried out by Sherman (1933) in U.S.A. and by Hastings and Newton (1934) in British Columbia that they are very resistant to heat treatment and require longer than 20 min. to kill them in water at 110° F. If they are previously soaked in water so as to render them motile they can be killed in a shorter time than if dry. Revival of any quiescent forms is therefore obviously desirable, and pre-soaking of bulbs for 24 hours before hot-water treatment with this end in view may be recommended if it will render the process more efficient. It is interesting to note that Courtney and Latta (1934) claim that in water at temperatures of 70–80° F. revival of quiescent forms is more complete than below 60 or above 100° F. Another point which emerges from this recent research is the desirability of early rather than late treatment of bulbs, *i.e.* before there has been time for the production of eelworm wool at the base of bulbs. Hastings and Newton (1934*a*), too, have shown that it is by no means a simple and easy matter to determine whether a given lot of eelworms treated by hot water has actually been killed when left in water to test for revival. Revival of motility apparently depends largely on the depth of the liquid in which the sample of eelworms is placed, for greater numbers revive in shallow than deep water. Also the amount of surface of the water exposed to the air is an important factor in inducing motility, and this must be related to the oxygen requirements of the worms, for it was found that the replacement of the air by carbon dioxide over the dishes containing treated worms for 20 hours prevented any return of motility.

I have already mentioned the desirability of early rather than late treatment of bulbs, but there are other factors to be borne in mind in determining the time of treatment. A very important one is that it should be applied during the period of the bulb's greatest dormancy. Hawker (1935), following up investigations by Gregory (1932), has confirmed his finding that bulbs treated in autumn may suffer heavily from attacks of the fungus *Fusarium bulbigenum.* She has also shown that bulbs treated during the period of maximum dormancy, *i.e.* during the latter part of August and in September, exhibit a phase of minimum susceptibility to *Fusarium* attack. It is clear, therefore, on these grounds, as well as because the bulb itself is now in the physiologically right condition, that it is desirable to give the treatment when the bulb is most dormant. One or two other points arising from Miss Hawker's work may be mentioned. She has shown that quick drying of the bulbs after treatment is to be recommended rather than leaving them in sacks to cool slowly, as is frequently done in this country. It is in no way harmful and is an additional precaution against possible attack by *Fusarium.* Another result is that the addition of formalin to the bath, so that the liquid is really a formalin solution of 0·1–1·5 per cent. strength, acts as a valuable fungicide and thus helps to reduce losses due to fungal attack. It has no harmful effects on the bulbs, either on the date of flowering, on the quality and number of blooms, or on the increase in weight of the bulbs during the growing season. We may expect to hear more in the near future, I think, on the suitability of other chemical substances for the treatment bath as lethal agents for nematodes and fungi.

Lastly, on this subject of hot-water treatment, I may briefly refer to recent work by Christie and Crossman (1935) on temperatures lethal to strains of *Aphelenchoides* parasitising begonias, chrysanthemums and strawberry. In the case of those causing disease in begonia leaves they found that 20 min. at 112° F. was sufficient to effect a complete kill of fifty specimens of the parasite. For worms from chrysanthemum leaves 12–15 min. at 115° F. was effective, 1½ hours at 112° F., but as long as 3 hours at 110° F. was required. One may point out in passing the difference between this time and that which has been recommended in this country for the treatment of chrysanthemum stools where as a result of the work of Hodson (1933) 20–30 min., according to size of stool, at 110° F. is claimed as effective in ridding the plant of the pest. The dangers attendant on heating stools to temperatures of 115° F. or slightly over in checking or retarding shoot production has been brought out by Kearns and Walton (1934), so that it would seem advisable to follow the recommendations of English workers in carrying out hot-water treatment of chrysanthemums even though there may be some slight risk of the incomplete destruction of all the parasites at a temperature of 110° F. for 30 min.

In the case of *Aphelenchoides* from strawberries, Christie and Crossman worked with material from two different parts of U.S.A., one from Massachusetts and one from North Carolina. Whereas a temperature of 115° F. for 10–12 min. was sufficient to kill the worms from Massachusetts plants, it required 55 min. at 118° F. to kill the worms from North Carolina plants. The disease symptoms and their seasonal occurrence differ in the plants from these two districts, and the authors are inclined to the view that the differences in response to hot-water treatment support the conclusion that two physiologically distinct strains of the parasite are concerned with attacks on strawberries in the two districts.

THE ROOT-KNOT EELWORM, *HETERODERA MARIONI*

I now turn to the consideration of certain matters connected with the root-knot eelworm, *Heterodera marioni*. In this country it is chiefly important as a pest of greenhouse crops such as cucumbers and tomatoes and of ornamentals such as begonias and cyclamen. There are also one or two records of it causing galls on the roots of plants in the open in this country, for example, on carrots in Wilts., parsnips at Slough in Bucks, and recently on celery in Co. Cork, Irish Free State. As soil temperature is one of the principal factors controlling the activity of this parasite it is interesting to note that it has been found attacking the roots of plants growing out-of-doors in more northerly climes than ours, for example on elder, snowdrop-anemone, chrysanthemum, paeony, iris, winter aster, cabbage and melon in Denmark. In Germany, Goffart (1934) has reported it from red clover, lucerne, peas, potatoes, vetch and sainfoin growing in the vicinity of Berlin. It would appear, however, to cause comparatively little damage to plants growing in these more temperate regions and is mainly a serious pest of out-of-door crops in warmer parts of the world. In many tropical and subtropical countries where valuable crops are cultivated the root-knot eelworm is one of the most serious factors limiting profitable production. Thus Scott (1934) recently reported that in California much of the land is limited to the cultivation of root-knot resistant crops or such as mature early in the season. In his opinion this parasite is the most serious in the State. In Nyasaland and Carolina it is a particularly serious pest of tobacco, especially in seed beds, whilst in Hawaii it is a great hindrance to the cultivation of pineapples. I have merely singled out one or two examples for special mention, but the range of hosts of cultivated and wild plants is very large, now reaching over 1000 names, including many weeds which in warm countries can serve as reservoir hosts on which the parasite can flourish in the absence of a susceptible crop. As a consequence the problem of eradication is rendered additionally difficult, and any attempt to grow immune or resistant crops in a rotation must entail very careful and frequent cultivation of the soil aimed at weed destruction. Control of the pest under greenhouse conditions can be effected by steam sterilisation of borders which, though expensive, is efficient, and to a lesser degree by chemical means. Under open field conditions control is much more difficult to achieve. The use of chemicals has been extensively investigated, and it is obvious that any substance recommended must be reasonably cheap for application on a large scale. So far chemical control has on the whole proved inefficient, but it is, of course, possible that someone will yet bring to light the ideal substance. Mention may be made of one substance which has given fairly good control in Hawaiian pineapple fields, namely, chloropicrin. It is very poisonous, and men injecting it into the soil have to wear gas masks. Also, in order to ensure good results it is necessary to check the escape of the vapour from the soil by covering the surface of plots with large sheets of tough paper which has been sized with glue. These are held down at the sides and the gas is left to act for about 4 days. With applications at the rate of 160–180 lb. of chloropicrin per acre reductions in the incidence of galling on roots in the region of 90 per cent. and an increased yield of pineapples round about 50 per cent. as compared with heavily infected areas have been effected. Chloropicrin when properly applied not only helps to eliminate the parasite but has a marked stimulating effect on the plant by increasing the vigour both of root and shoot growth (*vide* Godfrey, 1935).

Fallowing of infected soil has also been recommended, and mention may be made of the results of experiments conducted in South Africa by le Roux and Stofberg (1935), where for several months it was possible to expose the soil to desiccating conditions coupled with intense sunlight. Some of their experimental plots were ploughed monthly, followed by weeding, with the result that there was almost complete eradication of the pest. That frequent stirring and cultivation of the soil to assist in drying is an important factor in promoting destruction of the parasite is borne out by the extensive experimental work of Godfrey, Oliveira and Gittel (1933), who carried out investigations in Hawaii on the survival of the parasite as eggs, larvae or in affected pineapple roots under various conditions of dryness. They show that complete eradication of eggs and larvae can be effected in from 16 to 20 weeks if the soil is dried, accompanied by weekly stirring. If the soil is alternately wetted and brought to dryness, accompanied by stirring, eradication of eggs and larvae is effected in a somewhat shorter time. When the parasite is present in pineapple roots, drying with stirring brings about destruction in about 20 weeks. Exposure of galled woody roots of pineapple to bright sunlight for 7 or 8 days is sufficient to destroy the parasite within.

It would appear then that where climatic conditions are favourable, fallowing of infested land accompanied by frequent cultivation and weed destruction may provide an effective method in eradicating the pest. There are risks attendant on such operations, and it has been suggested that soil exposed to these conditions is liable to suffer seriously by the loss of its valuable humus content and by the destruction of beneficial bacteria. Again, it may be eroded if left too long without crops. This naturally leads to the consideration of methods of control which provide against such risks by the cultivation of rotational crops resistant to attack which can be used as green manures and to maintain the fertility of the soil. The chief of these are cereals, used as winter crops, followed by maize or some kind of immune legume as a summer crop, or by a mixture of these. As long ago as 1923 Godfrey gave particulars in one of the *U.S. Farmer's Bulletins* (No. 1345) on the best way of conducting a 2- or 3-year rotation along these lines. The same worker (1928) has also dealt with the problem of the most suitable of these immune legumes as rotation crops for pineapple fields in Hawaii. It would appear that Mauritius and Florida varieties of the velvet bean (*Mucuna* sp.), Laredo soy bean, certain varieties of cowpea, namely Iron, Brabham, Victor and Monetta, Florida beggar weed (*Meibomia tortuosa*) and Sunn hemp (*Crotalaria juncea*) fulfil most of the requirements in this direction. It is claimed that when rotations including a selection of these crops are carefully carried out the parasite is starved out and the soil is enriched rather than impoverished.

Immune or highly resistant crops can be recommended, however, not only for rotations but as principal crops. In the United States a good deal has been done on this question, and in addition to the cereals, maize and immune legumes already mentioned there are hybrid tobaccos which have proved resistant as well as certain named varieties of sweet potato whose use can be recommended. American grapes are more resistant than Old World varieties, and certain figs are less susceptible than others. Peach orchards in southern parts of the United States suffer particularly from the parasite, but in a note recently published by Auchters (1935) mention is made of the Shalil peach, originally imported from India, which has proved quite resistant to attack and is now being used extensively as a stock on which other susceptible peaches are being worked by grafting. Enough has probably been said to indicate the difficulties of control in the case of this parasite in warm countries and to show that by carefully planned rotations with the use of immune crops or by dry fallowing the problem is not entirely hopeless and insoluble.

HETERODERA SCHACHTII PROBLEMS

I would now like to say something on one or two aspects of problems connected with *Heterodera schachtii*. The original sources of infestations with this species will possibly never be fully elucidated in view of the fact that we have to reckon with five more or less fixed biological races of the parasite. These are: (i) the potato strain which is limited to attacks on potato, tomato and black nightshade; (ii) the beet race which will attack a fairly wide range of hosts including chenopodiaceous plants and several crucifers besides a variety of weeds; (iii) the oat race which confines its attentions to cereals and grasses but has also been found to attack certain weeds and red clover; (iv) the pea strain which will attack peas, vetches, broad beans and red clover; and (v) a race occurring in Japan which is specialised to the soy bean but will also attack kidney bean, adzuki bean and multiflora bean. It is not my intention to enter on a general discussion of the subject of biological races at this stage of my address but to consider certain aspects of the problem of the sources of infection in relation to such matters as clean seed, weed eradication and crop rotation. A crop of potatoes, sugar-beet, mangolds or peas is found to be doing badly, and when the roots are examined they are found to show various abnormalities and to have numbers of the little cystic females of *H. schachtii* attached to them. One naturally asks what was the source of the infection. It is generally extremely difficult to give a satisfactory answer to such a question. In the case of the pest on potatoes I can make no suggestion as to what may have been the original host of this now highly adapted race; possibly some wild solanaceous plant. We do know, however, something about its dispersal. The brown cysts may be carried on "seed" potatoes and in the small quantities of soil which get bagged up with them. When the sacks of seed are taken on to fields and the setts are planted it is easy enough to see how foci of infection may become established. In the same way tomatoes grown in potato-chitting houses become infected from the cysts introduced with the setts and soil. There is evidence also that the parasite can be carried on the seed coats of sugar-beet seed. In fact it has for many years been assumed that it is by such means that the pest has been spread to previously clean centres. My colleague, Dr Triffitt, has recently shown (1935) that in a small quantity of imported sugar-beet seed, which had been distributed to an English farmer by a sugar-beet factory, there was a certain amount of soil which, on being extracted with water, yielded a typical lemon-shaped cyst of the parasite. The possibility of dispersal by means of seed suggests the need for the establishment of regulations governing the importation of foreign seed to ensure its being clean and free from soil. It is quite probable that in various centres in this country where the pest has already been found attacking sugar-beet and mangolds, one source of origin has been its introduction by means of dirty seed. Once in the soil it has rapidly established itself under conditions favourable to its multiplication in the frequent cropping with sugar-beet or mangold which has been too often practised. It has also transferred itself to certain weeds commonly occurring in arable fields. Thus Triffitt (1929) found it in a field in the west of England in which mangolds were heavily attacked, on the roots of cauliflowers, mayweed, sow thistle, dead nettle, annual and perennial stinging nettle, poppy, black nightshade, speedwell, couch-grass, perennial rye-grass and annual meadow-grass. On none of these was there a heavy infestation, but it is easy to see how the parasite could maintain itself on a wide range of weeds during the cropping with intermediate crops in the absence of mangolds.

In the United States, also, it has generally been assumed that the parasite was introduced by means of infected seed, and this, of course, may have been the case in some instances. It is interesting to note, however, that in Utah, where sugar-beet is extensively grown and where beet sickness due to the parasite has been known for some years, the pest, according to Steiner (1935) and Thorne (1935), has recently been found on the roots of an indigenous weed known as shadscale. This is a species of the genus *Atriplex*, *A. confertifolia* (Torr. and Frem.) S. Wats., a member of the Chenopodiaceae, *i.e.* the same natural order as sugar-beet. This infected weed occurs in the Utah desert regions where sugar-beet has never previously been grown, and it is highly probable, therefore, that the widespread infestations of sugar-beet which have occurred in Utah have originated by transference of the parasite from the weed to the crop. A discovery of this character is not only of interest as to the origin of the pest but is of immediate practical importance, as it shows the necessity of excluding sugar-beet as a crop from areas where the weed occurs and of eradicating the weed as completely as possible so that it may not serve as a reservoir host of the parasite.

We may, I think, quite reasonably posit the occurrence of natural infections of the parasite on various weeds and grasses in this and European countries as the original sources of infection of cultivated crops, just as we know of the occurrence of the stem eelworm on many weeds. We do, in fact, know of the occurrence of natural infections on Marram grass in Devon and Aberdeenshire, and it is feasible to suggest that the infections which have been recorded on the roots of grasses on golf courses and bowling greens may have been derived from such a source, since seaside turf is often used for these purposes.

Triffitt (1929 and 1929*a*) has twice found it on couch-grass, once in a field where mangolds were affected and once where potatoes were attacked. In the latter case, however, the female cysts were not of the same shape as those of the potato race but were typically lemon-shaped, *i.e.* like those of the other races of the parasite. They may have been there as a natural infection or have remained there from some earlier infestation of mangold, beet or oats. Or again, they may have originated from the potato race, but this last suggestion is highly improbable seeing that attempts to transfer them experimentally from couch-grass to potatoes completely failed. Carroll (1933), working in Ireland, also failed to get transference of the parasite naturally occurring on dock to potatoes, nor did he succeed in effecting transference in the opposite direction, though both dock and potato were found heavily infected in the same field. Here are two instances of the parasite occurring on two common weeds, but whether they were original natural infections or had transferred from some cultivated crop cannot, of course, be ascertained.

The problem of the origin of the oat race of the parasite is more baffling, for we can scarcely suppose that this has been spread from place to place by means of infected seed. In this case I think we must turn to the possibility of its occurrence as a natural infestation on grasses, whence it has transferred to cereals. I have already mentioned that Triffitt twice found cysts of the parasite on couch-grass and once on perennial rye-grass. Perennial rye-grass has also been found by Edwards (1935) to be attacked by the oat race under experimental conditions, and he has also recorded (1935*a*) a severe attack on this grass in a first-year ley in Shropshire, presumably by the oat race. The same kind of grass has been found parasitised on a bowling green in Yorkshire by Thompson (1935), and it would seem that it is susceptible to attack by both the mangold and the oat strains of the parasite. It would, of course, be unwise to suggest that it is probably the original natural host of the parasite, but at any rate, the facts here adduced are suggestive of the direction in which we may expect to find the solution of this problem. In South Australia, where oats and wheat have been severely attacked by this parasite, Johnston (1934) has shown that it occurs also on the roots of two species of grass commonly found as weeds, namely, wall barley (*Hordeum murinum* L.) and sterile brome-grass (*Bromus madritensis* L.). Here, again, it is possible that we have the original hosts from which the parasite has transferred to cultivated cereals. In the case of the race attacking peas, the occurrence of which has been recognised in numerous scattered centres in England in the last few years, we cannot reasonably imagine it as being seed-borne. It seems more feasible to surmise the occurrence of natural infections on some wild vetch, since we know from experiments that this strain will attack the common vetch.

I have probably said enough to illustrate the wide implications of these problems, the puzzling questions which they raise and their great importance in such matters as crop rotation and the possible role of weeds as reservoir hosts.

CONCLUDING REMARKS

The many-sidedness of the incidence of eelworm diseases of plants will have become apparent from what I have already said. The varied character of the problems involved in attempts to establish control of diseases due to such intractable organisms need scarcely be emphasised. It is also obvious, I think, that final success in the campaign against such diseases is not likely to be achieved by the extreme specialist on eelworms, but will probably be the outcome of efforts put forth by many workers including the specialist on nematodes, the agriculturist or horticulturist, the chemist and the plant breeder. Investigations into many aspects of these problems are in progress in various parts of the world, though the number of workers engaged on them is comparatively small. So far as I know, however, nowhere is there provision made for instruction on these matters, and anyone taking up work on eelworm diseases of plants is for the most part thrown back on his own resources and on what he can glean from the literature. This brings me to the final part of my address.

In what I am about to say concerning the general lack of provision for instruction on plant helminths at universities and other centres of higher education, I doubtless lay myself open to the criticism that, being a specialist, I tend to overestimate the importance of the subject in the whole body of applied biology. Whilst admitting whatever validity there may be in such criticism, I still think that the present position is to be regretted and needs remedying. Perhaps we cannot expect that in courses for an honours degree in zoology more time should be devoted to the helminths in general, since they form but a comparatively small and mixed assemblage of the invertebrates. Moreover, we have to remember that the real advances in our knowledge of helminth bionomics have been made not by academic zoologists but by parasitologists and other specialist investigators interested in the applied aspects of parasitology. Again, the teaching of zoology is to-day in the main "pure" as contrasted with "applied". Indeed "applied" is apt to be regarded as a little suspect: interesting perhaps as illustrating the way in which zoology impinges on disease in humans or in domesticated animals and plants or on the many varied ways in which animals and plants are of use to man, but not to be studied too deeply, since it does not relate particularly to the main scheme of the zoological curriculum which is designed to display the form and function of the chief groups of the animal kingdom and their relatedness to one another in a general evolutionary scheme. In taking this somewhat critical attitude towards current academic teaching of zoology

I range myself with my predecessor as President of this Association and fully subscribe to the criticisms of the present-day academical outlook which he so admirably set forth in his Presidential Address.

The needs of the medical and the veterinary student in helminthological instruction are being fairly well met at the present time, and for post-graduates intending to take up tropical medicine there is ample provision. For anyone, however, taking up agriculture or horticulture no provision is available, and we badly need a course in "nematology", to borrow a useful word coined by Dr Cobb. From time to time we recruit men for the agricultural and horticultural services as advisory or consultant officers. At the annual meeting of the Association of British Zoologists held early in January last, Fryer discussed the question of the qualifications required by men who are likely to succeed in this work. Here are some of his points. They should have obtained a good degree in the biological sciences, *i.e.* they should combine a knowledge of zoology and botany and, also, should have some knowledge of chemistry. They should have personality and an ability to appreciate sympathetically the farmer's or fruit grower's point of view; which means that they ought to know something about farming or horticulture. They should have an aptitude for natural history. All things considered, the countryman rather than the townsman seems to be indicated. Given the right type of man, qualified in the ways just indicated, we equip him further by specialised instruction in agricultural entomology or, in the case of a botanist, in mycology, but there we stop and omit to give instruction in the veriest rudiments of nematology. My suggestion is that for such people a short course covering general helminthology should be devised, and they should learn something on the technique of collecting and examining eelworms, the characteristics of the chief parasitic species attacking plants of economic importance and the symptoms to which they give rise, the features by which the parasites may be differentiated from the commoner saprophagous forms and methods of control.

We know that men in such consultant service are bound sooner or later to encounter diseases of agricultural or horticultural crops caused by nematodes and will be asked to give advice on them, yet we are at present content to turn them out for their work without any equipment with which to cope with the eelworm problems they are likely to meet. It seems to me desirable that before these men embark on their work in the agricultural services we should make provision for their proper training in matters connected with nematode diseases of crops.

In university courses where a student takes an honours degree in agriculture, and particularly where he has an opportunity of taking zoology as a subsidiary subject, his courses should include some instruction in agricultural parasitology covering not only helminths of farm stock but of agricultural crops as well. Similar suggestions would apply to the advanced student of horticulture, for it is surely not proposing too much to suggest that he should be equipped with some knowledge of the important diseases of horticultural crops caused by eelworms. I believe I am right in saying that, at the present time, there is no university, agricultural or horticultural college in the world which provides for such instruction as I have attempted to show is so much to be desired.

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THE QUANTITATIVE DISTRIBUTION OF BORON IN *VICIA FABA* AND *GOSSYPIUM HERBACEUM*

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Introduction

Beginning with the well-known work of Warrington (9) in 1923 and Brenchley and Warrington (2) in 1927, a considerable amount of work has already been done upon the boron requirements of certain plants, with regard to the morphological and anatomical and, to a lesser extent, the physiological effects consequent upon variation in the boron dosage. A wider survey by Sommer and Lipman (7) showed that boron requirement was very widespread among angiosperms. Its importance, if not its essential character, as an element of plant nutrition, in at least a large number of species, may be regarded as firmly established.

Boron starvation leads to definitely pathological symptoms. On the other hand, there is some evidence (Johnston and Dore (3), Morris (5)) that, within limits, growth may be quantitatively related to the amount of boron present, which points to the absorption of boron by the plant.

The present work starts from this point, and is an attempt to push the question a stage further by quantitative determination of the distribution of boron in the tissues. Such information may act as a check upon physiological speculation and provide data for experimental investigation.

The amount of boron tolerated in culture solutions is so small, lying between limits of the order of $0{\cdot}5 \times 10^{-6}$ and $0{\cdot}5 \times 10^{-3}$, that it is obvious that only a very delicate analytical method will serve, even though some concentration of boron in the tissues is to be expected. It is, therefore, necessary to use plants which have a high boron tolerance, and to increase the concentration supplied as far as possible. The broad bean, *Vicia Faba*, is the classic plant of Warrington's first researches, and was chosen as the chief experimental material, but this was supplemented by some analyses of *Gossypium herbaceum*, which has an even higher boron tolerance than *Vicia*. As microchemical tests are not applicable in this case, analyses have been made of alkaline extracts of ashed tissues by a modification of the very sensitive colorimetric method of Cassell.

EXPERIMENTAL METHOD: EXTRACTION

When a solution of curcumin, or turmeric acid itself, is added to a comparatively dilute solution of boric acid or borax, an intense red coloration is produced, which decreases in intensity with dilution, passing through orange, pink and yellow and gradually disappearing. A more intense coloration is obtained when boric acid reacts with oxalic acid and curcumin in the dry. The method was devised by Cassell in 1903, and is well suited for the estimation of boron in small quantities. Concentrations of boron trioxide as low as 0·000002 gm. (2γ) per c.c. can be estimated by this method, full details of which are given by Cassell in the *Chemical News* (1907).

The method was intended by Cassell to determine the amount of boron in foodstuffs. It proved unsatisfactory, in its original form, for the estimation of boron in green plants, and the following method was elaborated instead.

The plants or plant organs are very carefully washed free of all adhering soil material. To ensure thorough removal of the soil the plants were allowed to soak for a few days. Great care must be exercised to avoid injury or death of the plants at this stage, since a loss of boron by exosmosis would be involved. The plants are then carefully dried with filter paper and desiccated to constant weight in an air-oven at a temperature of 140° C. A known quantity of the material was then taken for the estimation of boron. Following Cassell's original method, it was found that, when the material is made strongly alkaline with barium hydroxide, then evaporated to dryness and the residue acidified with HCl and extracted with several successive portions of hot water, the filtrate obtained was darkly coloured by decomposition products of chlorophyll, etc. This dark colour could not be removed with animal charcoal, since the latter would adsorb the boron. Therefore the dry material was rendered strongly alkaline with a saturated solution of barium hydroxide, gently evaporated over a Bunsen flame to dryness and then more strongly heated to decompose the organic substances and remove the carbon. Cassell discovered that, in spite of the low volatility of boric acid, there is an appreciable loss in evaporation and in ignition unless barium hydroxide is added. Too intense heat results in a loss of inorganic matter, but the carbon must be entirely removed, since it interferes with the acid extraction of mineral elements in the ash.

The ash is extracted with 24 per cent. HCl. The crucible is then washed out with dilute HCl to remove adhering traces of mineral matter, and the acid solution is made up to 100 c.c. Of this solution 10 c.c. are used in the following manner.

The 10 c.c. are transferred to a clean, dry evaporating dish. An alcoholic solution of curcumin (0·25 gm. in 250 c.c.) is then added drop by drop, until the further addition of one drop no longer produces any increase of red colour. Then a saturated aqueous solution of oxalic acid is added, drop by drop, till the red coloration disappears. Boron is present in the root in such minute quantities that no red colour is obtained when curcumin is added to the acid extract. In this case curcumin should be added till no further yellow colour results. Two to three drops are usually sufficient and ½ c.c. of oxalic acid is then added, with 15 gm. of silver sand, which has been extracted with hydrochloric acid for 2 days and thoroughly washed and dried. The contents of the dish are evaporated on a water-bath.

It should be emphasised that the experimental error increases with the number of stages in a reaction. Cassell's original method at this stage is laborious and inconvenient, and comparison of the two methods showed that evaporation on a water-bath is the more satisfactory of the two. On the other hand, it must be borne in mind that the reaction between B_2O_3, curcumin and oxalic acid occurs at the drying point. It is necessary, therefore, to ensure thorough mixing, intimate contact, and gentle evaporation. A watch-glass is placed over the dish to prevent loss through spitting. The red pigment in the residue is of unknown constitution, but is probably very complex. It is finally extracted with 70 per cent. alcohol and the amount made up to 100 c.c.

EXPERIMENTAL METHOD: COLORIMETRIC DETERMINATION

A standard solution of known concentration is prepared. 2·886 gm. of $Na_2B_4O_2 . 10H_2O$ is equivalent to 1·0 gm. of B_2O_3. If this quantity of borax is dissolved in a litre of distilled water, then each c.c. will contain 0·001 gm. of B_2O_3. Of this standard solution 10 c.c. are treated in the manner described above and the red colour extracted with 70 per cent. alcohol, and made up to 100 c.c. as before.

Preliminary comparison is effected by dilution of the extract from the standard, till the intensity of colour is a little less than in that from the unknown solution. The solutions must be compared in test-tubes of equal cross-sectional area, against a white background. It should be noticed that Cassell's method of adding a drop or two of curcumin at this stage to equalise the tints is unsatisfactory. Great care must be taken to add just enough at the initial stage, since it is extremely difficult to compare different tints of different intensities. Accurate determinations of the intensities were made with an Autenrieth-Hellige wedge colorimeter, which consists essentially of a rectangular receptacle to hold the standard solution and a glass wedge running on a scale (representing the depth of solution in the wedge, in millimetres ÷ 10) to hold the unknown solution. Two samples of the standard are themselves first matched and the reading observed. The wedge is thoroughly dried and filled with the unknown solution, which should be a little stronger than the standard. The point at which they match is noted and the weight of B_2O_3 per c.c. of the unknown solution is then determined in the following manner. Suppose that the diluted standard contains 0·0001 gm. of B_2O_3 per c.c. and matches with itself at 12·1 mm. on the colorimeter scale, and that it matches with the unknown at 7·3 mm. Then the weight of B_2O_3 in grams per c.c. of the unknown solution

$$= \frac{0{\cdot}0001 \times 12{\cdot}1}{7{\cdot}3} = 0{\cdot}0001658 \text{ gm./c.c.}$$

The weight of B_2O_3 in the original solution may then be readily determined for purposes of comparison as a percentage of the original dry weight of the plant material.

In concluding these observations on the experimental method, it is necessary to emphasise that scrupulous care must be taken to avoid contamination with boron from an extraneous source. All the chemicals used were of the B.D.H. standard of A.R. purity, and were all tested for the presence of boron with negative results.

TESTS OF THE EXPERIMENTAL ERROR

The experimental error involved in the process was determined in the following manner. Ten c.c. of a solution, containing 1 part of boron trioxide (B_2O_3) in 1000 parts, were diluted to 100 c.c., and 10 c.c. of this solution were operated upon in the usual manner. The red precipitate obtained was dissolved and made up to 20 c.c. The operation was repeated on another 10 c.c. and the red solution obtained again made up to 20 c.c. Each c.c. of these solutions should contain 0·00005 gm. of B_2O_3 per c.c., whereas, by comparison with solutions of known concentration they were found to contain respectively 0·0000482 gm. of B_2O_3 c.c. and 0·0000448 gm. per c.c. These two observations show an average error of 7 per cent. (less than one integer) in the fifth decimal place. In the following tables the weights of boron found are, therefore, treated as significant to four places. They are to that extent also consistent among themselves.

CULTURAL METHODS AND CONDITIONS OF GROWTH OF PLANTS

In order to evaluate the effect of varying concentrations of boron in the medium on its distribution in the plant, the plants were all grown in sand. The sand was first washed very thoroughly with tap water and then with distilled water. It was also sterilised by steaming for a half an hour on three consecutive days. The sand was placed in unglazed pots which had also been thoroughly cleansed. The plants were watered daily with the following three-salt solution.

Weight-molecular solutions were made up of potassium hydrogen phosphate (KH_2PO_4), calcium nitrate ($Ca(NO_3)_2$) and magnesium sulphate ($MgSO_4$). From these, 180 c.c. of KH_2PO_4 solution, 50 c.c. of $Ca(NO_3)_2$ solution, and 150 c.c. of the $MgSO_4$ solution were mixed and made up to 10 litres with water. A suspension of ferric phosphate in the proportion of 2 gm. per 100 c.c. of water was added at the rate of 10 drops to each litre of solution. The seeds were germinated *in situ* in the sand.

The culture pots of each species used were divided into two lots. One lot was watered, in addition to the above, with a solution of borax containing B_2O_3 in the proportion of 1 in 5000 (0·02 per cent.), and the other lot with an equal amount of a solution of borax of a concentration of 1 in 50,000 (0·002 per cent.). The plants were maintained at an even temperature in an electrically heated and regulated culture chamber in a greenhouse.

Whereas boron trioxide at a concentration of 1 in 5000 proves fatal in water culture, plants grown in sand, when watered with solutions of this concentration, show healthy growth, until the plant is six nodes or so high. The cotton plants, however, died before the third node was developed, when grown in 1 in 5000 B_2O_3, even in sand.

EXPERIMENTS AND RESULTS

A. Vicia Faba, *watered with* 1 *in* 5000 B_2O_3

Experiment 1.

Four lots, A, B, C and D, of healthy plants, six nodes high, were dried at 110° C., and each lot divided into three fractions: leaves, stems and roots respectively. These were ashed, separately, and operated upon as outlined above. The weight of boron present is expressed in the third column of Table I, as a percentage of the dry weight.

Taking each type of organ separately, the average percentage contents of B_2O_3 are: *Leaves*, $0{\cdot}225 \pm 0{\cdot}012$; *Stems*, $0{\cdot}045 \pm 0{\cdot}0029$; *Roots*, $0{\cdot}003 \pm 0{\cdot}0002$ (standard errors in each case). The last figure is probably too small to be reliable, though the four analyses agree well together. The amount in the root is, at any rate, of a much smaller order than in either stem or leaf.

Table II gives the total dry weight of the plants in each lot, the total weight of boron trioxide in the plants, and the corresponding percentage of boron present in all organs taken together.

Table I

	Organs	Dry wt. in gm.	Wt. of B_2O_3 in gm.	B_2O_3 %
A.	Leaf	4·980	0·0105	0·210
	Stem	7·670	0·0035	0·050
	Root	2·730	0·0001	0·003
B.	Leaf	5·634	0·0133	0·240
	Stem	8·367	0·0043	0·050
	Root	2·912	0·0001	0·004
C.	Leaf	7·536	0·1509	0·200
	Stem	11·328	0·0043	0·040
	Root	3·864	0·0001	0·003
D.	Leaf	9·132	0·0233	0·250
	Stem	13·458	0·0056	0·040
	Root	4·345	0·0001	0·003

Table II

	Total dry wt. of plants in gm.	Total wt. of B_2O_3 in gm.	Average B_2O_3 %
A.	15·380	0·0141	0·092
B.	16·913	0·0177	0·105
C.	22·728	0·0207	0·091
D.	26·935	0·0292	0·108
Totals	81·956	0·0817	0·396

The average percentage weight of boron trioxide for the dry weight of the whole plant is 0·099 ± 0·005 per cent. (standard error).

From this preliminary experiment it is obvious that boron is stored in the leaf tissues. The concentration of boron in the leaves is over five times the concentration in the stem.

The concentration in the root is very low, so low that it is very difficult to estimate accurately, and the results lie within the limits of experimental error.

Experiment II. Estimation of boron in primary and secondary roots.

The roots were very carefully raised from the soil and washed. They were then divided into two lots, one lot consisting of primary roots and

Table III

Organs	Dry wt. in gm.	Wt. of B_2O_3 in gm.	B_2O_3 %
Primary roots	3·567	0·0001	0·003
Secondary roots	1·230	0·00005	0·004

another lot of secondary roots. These were operated upon in the usual manner, and the resulting solutions were compared with the standard solution (Table III). The difference found is probably not significant.

Experiment III. Estimation of boron in the petiole.

Three samples of petioles, including the stipules, were dried, ashed and operated upon as usual. Analysis by comparison with the standard solution gave results shown in Table IV.

Table IV

Dry wt. in gm.	Wt. of B_2O_3 in gm.	B_2O_3 %
3·285	0·0028	0·087
5·165	0·0040	0·078
2·372	0·0016	0·070

The concentration of B_2O_3 in the petiole is higher than the concentration in the stem. It is intermediate in value between the leaf and stem concentrations.

A number of experiments were performed to obtain data on the distribution of boron in the stem alone.

Experiment IV. Concentration of boron in the stem apex.

The apices of a number of healthy plants were removed, dried, ashed and operated upon. The results shown in Table V were obtained.

Table V

Wt. of apices in gm.	Wt. of B_2O_3 in gm.	B_2O_3 %
0·622	0·0006	0·098
1·238	0·0010	0·083

The concentration of boron in the shoot apex is almost exactly twice the average concentration of boron for the whole stem. Therefore, there is either an ascending gradient of boron in the stem or a local concentration of boron in the apex.

Experiment V. Gradient of boron in the shoot.

The amount of boron in successive nodes and internodes was estimated. A number of healthy plants about six nodes high were selected. The shoots were divided into five fractions. The first fraction consisted of the first node and internode below the apex of each plant. The term "node" is here meant to include a pair of leaves at the node with their accompanying stipules.

The second fraction consisted of the second node with its accompanying leaves and the internode below.

The third fraction consisted of the third node, leaves and internode and so on.

These five fractions were separately dried, ashed and estimated, with results summarised in Table VI.

Table VI

No. of fraction	Wt. of fractions in gm.	Wt. of B_2O_3 in gm.	B_2O_3 %
1	2·653	0·0027	0·104
2	2·967	0·0037	0·124
3	3·446	0·0047	0·137
4	4·182	0·0066	0·159
5	5·039	0·0095	0·189

It appears that the concentration of boron is directly proportional to the age of the node and the subtending leaf. These results tend to disprove the hypothesis that there is a regular diminution of boron in the stem from apex to base. There is a concentration of boron in the apex, but otherwise the distribution in the shoot shows a regular basiscopic increase of concentration.

Experiment VI. Estimation of boron in the seed of Vicia Faba.

A number of seeds of average size, from plants grown in ordinary soil were ground up, dried, ashed, etc., and the amount of boron estimated as usual. Table VII summarises the results obtained.

Table VII

Dry wt. of seeds in gm.	Wt. of B_2O_3 in gm.	B_2O_3 %
9·236	0·0066	0·072
18·685	0·0011	0·063
26·862	0·018	0·068

The boron is confined to the cotyledons. Analysis of the seed coat gave negative results.

Experiment VII. Distribution during germination.

A number of broad bean seeds were germinated partially submerged in distilled water. The water was previously tested and was found to be devoid of boron. The tips of the plumules were removed as soon as they became green. If any boron is found in the tip then it must be derived from the cotyledons. Estimation of the tips gave the results shown in Table VIII.

Table VIII

Dry wt. in gm.	Wt. of B_2O_3 in gm.	B_2O_3 %
1·384 (plumule)	0·0003 (pl.)	0·035 (pl.)
7·658 (cotyledon) (after germination)	0·0028 (cot.)	0·036 (cot.)

There appears to be a migration of boron from the cotyledons to the plumule during germination. The average percentage of B_2O_3 in the seed is 0·068. The percentage in the plumule is 0·025. The total percentage in germinated seed and plumule only is 0·061. The remaining 0·007 per cent. is distributed between the lower portion of the plumular axis and the young radicle. The significance of this result will be referred to under "Conclusions".

The following experiments were conducted on plants of *Vicia Faba* watered with solutions containing 1 in 50,000 boron trioxide.

Experiment VIII. Bean plants in low boron concentration.

A number of healthy plants were divided up into three lots; lot (1) of leaves, lot (2) of stems, and lot (3) of roots. These lots were analysed separately, and gave the results shown in Table IX.

Table IX

	Organs	Dry wt. in gm.	Wt. of B_2O_3 in gm.	B_2O_3 %
A.	Leaves	3·634	0·0059	0·16
	Stems	5·825	0·0011	0·02
	Roots	1·962	0·0001	0·002
B.	Leaves	5·733	0·0070	0·12
	Stem	9·521	0·0013	0·01
	Roots	3·016	0·0001	0·002

Percentage of B_2O_3 *in whole plant grown in* 1 *in* 50,000 B_2O_3

Weight of plants in gm.	Wt. of B_2O_3 in gm.	B_2O_3 %
29·691	0·0154	0·05

Comparing this percentage with that found in Table II, we see that, although the relative concentration of the solutions was 1 : 10, the ratio of concentration in the tissues is only 1 : 1·9.

Experiment IX. Stem apex at low boron concentration.

The apices of a number of healthy plants, grown in 1 in 50,000 B_2O_3 were removed, divided into two lots, and ashed. The results obtained are shown in Table XI.

Table XI

Dry wt. in gm.	Wt. of B_2O_3 in gm.	B_2O_3 %
1·4652	0·0011	0·08
0·8753	0·0006	0·07

Comparing these figures with those in Table V, the ratio of concentration is seen to be 1 : 1·3 as compared with a solution ratio of 1 : 10. Both results point to a relatively greater absorption of boron from the more dilute solution supplied.

B. Gossypium herbaceum

Determinations of the distribution of boron in the cotton plant were also made.[1] The plants were grown, as before, in two different concentrations of B_2O_3, viz. 1 in 5000 and 1 in 50,000, but the plants growing in 1 in 5000 B_2O_3 did not thrive beyond the seedling stage.

Experiment X. Estimation of boron in cotton seed.

The seeds were crushed, thoroughly dried and then analysed as usual, the results being shown in Table XII.

Table XII

Wt. of seeds in gm.	Wt. of B_2O_3 in gm.	B_2O_3 %
2·535	0·0011	0·042
3·417	0·0013	0·036
5·258	0·0021	0·039

Experiment XI. Boron content of cotton seedlings.

The seedling consisted of the paired seed leaves, young shoot and radicle. The seedlings were grown in 1 in 5000 B_2O_3. They were divided into two lots and each lot into three fractions, leaves, shoots and roots, which were then analysed independently. The results are given in Table XIII.

Table XIII

	Organs	Dry wt. in gm.	Wt. of B_2O_3 in gm.	B_2O_3 %
A.	Leaves	2·780	0·0068	0·250
	Stem	2·152	0·0011	0·050
	Roots	1·630	0·0001	0·003
B.	Leaves	1·857	0·0046	0·250
	Stem	1·623	0·0010	0·060
	Roots	0·952	0·0033	0·003
	Totals A and B	10·994	0·0169	0·616

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[1] Seed of two varieties: American Upland, in the fifth home-grown generation, and Egyptian Sakellarides direct from the Sudan, was very kindly supplied by Prof. R. H. Stoughton.

The average percentage content for all organs taken together is 0·103 ± 0·002, which is slightly, though probably not significantly, higher than in the broad bean (0·099 per cent.).

Experiment XII. Cotton in low boron concentration.

A quantity of cotton plants, about three nodes high, was divided into three fractions, leaves, stems and roots. These plants had been watered with a solution of B_2O_3 of concentration 1 in 50,000. The fractions yielded the results shown in Table XIV.

Table XIV

Organs	Dry wt. in gm.	Wt. of B_2O_3 in gm.	B_2O_3 %
Leaves	2·980	0·0056	0·19
Stems	3·673	0·0012	0·03
Roots	1·876	0·0001	0·003

Comparing, as before, with the broad bean, the ratios of concentration found in the tissues with those in the nutrient solutions, we find that the ratio of boron concentration in the solutions was 1 : 10, while the ratio in the tissues is only 1 : 1·44, so that, as in the case of the bean, there is relatively greater absorption from the more dilute solution and that in about the same degree in both cases.

CONCLUSIONS

The foregoing results are presented by the authors simply as a work of analysis, for the provision of necessary data. While the spectroscope has shown the constant presence in living tissues of traces of elements not deemed essential in the past, the development of quantitative colorimetric methods of great delicacy will make it possible to get more information about the storage and utilisation of these "accessory" substances, especially aluminium and manganese.

Boron is absorbed in such exceedingly small amounts that it cannot be regarded as being on a par with the kations which form the regular mineral nutriment of the plant, potassium, magnesium, phosphorus, etc. It must act in some manner as an accessory substance, possibly as itself a catalyst, like manganese, or, more probably, as an activator or regulator of other catalysts.

In whatever manner it enters the plant, its internal distribution is perfectly definite. The highest concentration is in the leaves, increasing with the age of the leaf; the next highest concentration is in the petioles, which is almost the same as that in the stem apex, the rest of the stem having a much lower content and the roots the lowest of all. The percentage of boron in the older leaves rises so high that it is difficult to avoid the conclusion that it must be stored, at least in part, in an inert form.

In view of this fact it is curious that no one has recorded an effect of boron deficiency on leaves, though Johnston and Dore (3) and McHargue and Calfee (4) record marked necrosis of the stem apex and Sommer and Sorokin (8) pathological changes in the root apex as a result of boron starvation. The first named found leaf injuries due to boron excess, and they remark that there is an increase of dry weight in plants grown with boron, the increase being in the order leaf, stem, root.

The concentration in the seed cotyledons is intermediate between that of stem and leaf in the growing plant.

Such a distribution might be attributed to passive concentration by means of the transpiration current, were it not for two things. Firstly, the fairly high concentration in the bean cotyledons (average 0·067 per cent.) which is well above the concentration (0·045 per cent.) in the stem tissues, from which flower and seed are derived. It is well established that a developing secondary axis uses the subtending leaf as a source of sugar, and it seems probable that boron is, in this case, withdrawn from the leaf by the developing axillary flowers, and may perhaps be necessary before fruit can set. Secondly, there is the fact that in germination, part of this stored boron goes to the radicle and does not follow the transpiration path to the plumular apex. This indicates that diffusion must effect distribution, to some extent independently of transpiration.

Selective absorption is also indicated by the higher proportion of boron absorbed from more dilute solutions, so that, in some cases, the concentration in the tissues is almost as high in plants grown in 1 in 50,000 solutions as in those from 1 in 5000 solutions. A similar propensity is shown by the amount of boron in the seed (cotyledons) above referred to, which was found in stock seed, from plants grown in ordinary garden soil, in which the concentration of boron is very low, probably of the order of 1 in 100,000.

The steady increase of boron concentration, from the youngest leaves downwards, does not suggest the fluctuation which would be expected if boron were only a temporary occupant of the leaf cells, liable to translocation with other food materials to other parts of the plant. It rather suggests, on the contrary, that it is stored progressively in the leaves and in the apex of the shoot, the main centres of constructive metabolism. This progressive local concentration, again, does not accord with the idea that boron is a nutritive element in the ordinary sense, but rather with the view that it is related to the metabolic syntheses as an activator. There is some evidence to show that the C : N ratio in the tissues is reduced by the application of excessive amounts of boron and that this is due to reduction of carbohydrate formation, not to increased nitrogen assimilation, in spite of the fact, in the opposite sense, that boron starvation restricts the nodule formation on bean roots (Brenchley and Thornton (1)).

The basiscopic gradient also accords with Warrington's observation that older bean plants were less susceptible to excess of boron than young plants, which may perhaps be due to their greater carbohydrate reserves.

Schmucker (6) suggests that boron compounds with sugars, or other hydroxyl-containing substances, may enter into the formation of cell membranes. If this were so, the older stem tissues should show a higher boron content than the apex, as the older leaves have a higher content than the young ones, but the reverse is the case.

A connection between boron content and cambial activity is, further, suggested by the boron concentration in the apical tissues and in the leaves, which strongly induce cambial growth in the stem beneath them, and by the degeneration of vascular tissue which follows boron deficiency (Warrington (10)), as well as by the stimulus which it gives to nodule formation on the roots. The leaf effect on cambial activity is shown by plants in the dark and cannot, therefore, be due to photosynthetic products.

SUMMARY

1. The content of boron in the tissues of plants, grown in solutions of known concentration, has been ascertained in *Vicia Faba* and *Gossypium herbaceum*.

2. The distribution of boron is regular and definite. The highest percentage, per unit of dry weight, is in the leaves, increasing regularly with the age of the leaf. The petioles and the stem apex are approximately equal in percentage content, and have both approximately double the stem concentration. The roots have much the lowest concentration, only 0·07 of that in the stem.

3. This distribution of boron does not appear to be entirely due to passive transportation in the transpiration current.

4. Boron is also present in seed grown on ordinary soil, but is confined to the cotyledons, the percentage content, in *Vicia*, being 50 per cent. above that of the plant stems.

5. The amounts of boron absorbed are extremely small, though there is some storage in the tissues, and they are not directly dependent on the

concentration supplied. The amounts are too small to allow of boron being regarded as a nutrient in the ordinary sense. Its importance is more probably that of an activator or regulator of metabolic processes.

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THE BIOLOGY OF OAT SMUTS

IV. THE INVASION OF SOME SUSCEPTIBLE AND RESISTANT OAT VARIETIES, INCLUDING MARKTON, BY SELECTED BIOLOGICAL SPECIES OF SMUT. (*USTILAGO AVENAE* (PERS.)) JENS AND *USTILAGO KOLLERI* (WILLE)[1]

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(With Plates VII and VIII and 6 Text-figures)

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I. Introduction

In recent years, knowledge concerning the relationships between host and pathogen in the smut fungi has steadily increased, until now, the varied life cycles of species within this group of plant parasites are well known. The discovery of the widespread existence of biological specialisation has provided the opportunity for a more critical examination of host pathogen relationships, since a biological species will more nearly approach genetic purity than the taxonomic species as a whole.

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[1] This work, which was carried out under the direction of Miss K. Sampson while the writer was holding a Ministry of Agriculture Research Scholarship 1933–5, represents part of a scheme of work pursued at the University College of Wales, Aberystwyth, in preparation for the Ph.D. degree. Parts I–III of this series appeared in earlier numbers of this journal (see Sampson (13, 14, 15)).

Reed (9) in the United States and Miss Sampson (12) in Wales, working with *Ustilago Avenae* and *U. Kolleri*, proved the existence of distinct forms which differed in a consistent manner on selected varieties of oats. In work of this type, the presence or absence of smutted panicles is the criterion used for establishing the resistance or susceptibility of the host varieties, but it is now realised that plants which appear to be completely immune from attack, when judged on this basis, may yet contain living mycelium of the parasite (Brefeld (3), Kolk (6), Sampson (15)). Why this mycelium fails to produce spores in the panicles is not, as yet, understood. Brefeld held the opinion that it failed to develop at a rate sufficient to enable it to reach the young meristems before the surrounding stelar tissue hardened and so cut off access to them. It seems probable, however, that the problem is a complex one and is intimately linked with inherited degrees of resistance on the part of the host and of virulence in the fungal invader.

Brooks (4) has recorded the fact that oats in fields apparently free from loose smut sometimes throw up, after harvest, weak tillers, which are almost completely smutted, and Melchers (8) found that by cutting back healthy heads of sorghum plants he frequently obtained axillary shoots infected with kernel smut (*Sphacelotheca sorghi*). Melchers states that really resistant or immune varieties of sorghum do not react to mutilation in this way, and he therefore concludes that such plants do not contain mycelium in their tissues. In an earlier paper of this series, Miss Sampson (15) recorded results of a microscopical study of the progress of the mycelium of "virulent" and "non-virulent" forms of *Ustilago Kolleri* within the tissues of a selected line of *Avena strigosa*, showing that even an apparently resistant oat may carry the smut parasite in its basal parts up to a relatively advanced age (11 weeks). In the same paper it was suggested that oat varieties might possibly be classified according to their resistance to invasion by smut mycelium. The present work, which is a continuation of Miss Sampson's experiments, was undertaken with the object of obtaining further evidence bearing upon this question, and below are recorded results which suggest that definite "grades of resistance" do occur in oat varieties attacked by biological species of smut fungi. Within these grades, varieties have been found which range from total susceptibility to a resistance which almost amounts to true immunity.

II. MATERIAL AND METHODS

The experiments may conveniently be divided into two groups, namely:

(1) A study of two biological species of *Ustilago Avenae* attacking two host varieties so chosen that each could be examined in a resistant and a susceptible condition.

(2) The penetration and infection of the highly resistant variety Markton by six strains of loose and covered smut.

In the first group the two varieties of oats selected were (*a*) a selection[1] of the variety Potato (*Avena sativa*, Ref. No. 2855) and (*b*) a selection of Welsh Strigosa (*Avena strigosa*, Ref. No. 521). These were inoculated with two of Miss Sampson's collections of *Ustilago Avenae* to which she has given the serial numbers L_1 and L_{11}.

For the study of Markton, three forms of both *Ustilago Avenae* and *U. Kolleri* were selected. All of these spore collections had been kept under observation for ten years and had shown, with two possible exceptions, consistent and distinct infection capacities throughout.[2]

The procedure was as follows: Shelled grains of the oat varieties were liberally dusted with the appropriate spores and planted in sterile sand, with a moisture content of 20 per cent., in glazed earthenware saucers. These were weighed and placed in an incubator at 22° C., and any loss of weight due to evaporation was made up by the daily addition of water sprayed on to the surface of the sand. Under these conditions uniform germination and growth were obtained. When the leaves had emerged from the coleoptile in the majority of the seedlings, the dishes were removed from the incubator and allowed to remain on window ledges at the ordinary room temperature. According to Roesch (11) the cessation of susceptibility of the seedling appears to coincide with the emergence of the first leaf from the cotyledonary sheath, and since this did not take place until the young plant was from 5 to 7 days old, the time passed in the incubator was adequate for the germination of the smut spores and the penetration of the host. Seedlings were fixed in Navashin's fluid at 3, 7, 14 and 21 days, cut up and embedded in wax, and microtome sections $12\,\mu$ in thickness were cut and stained with a combination of Bismarck brown, gentian violet and Gram's iodine. With this stain the mycelium appears violet and the host cytoplasm brown, and in the case of the oat smuts differentiation is quite sharp and the hyphae are relatively easy to trace. From each batch of material a representative sample of plants was transplanted to boxes of soil and grown to maturity in order to determine the incidence of smutted panicles (Table I). The grains used were derived in each case from a single plant in order to exclude the possibility of contamination by rogues. The primary or coleoptile node was always examined and, in addition, blocks containing sections of leaves and coleoptile and mesocotyl were usually cut. The microscopical observations were made on three separate batches of plants sown on different dates, but, since these gave consistent results, it is unnecessary to deal with them individually.

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[1] These oats will be referred to in this paper as "Potato" and "Strigosa". The terms therefore only apply to these particular pure-line selections and not to the variety or species as usually understood. Letters L and C followed by serial numbers refer to forms of loose smut (*Ustilago Avenae*) and covered smut (*U. Kolleri*) respectively.

[2] Collections L_1 and L_2 have not maintained all their distinctive characters in recent years. The reason for this is not yet clear, but since these two collections reacted in a similar manner on Markton it does not affect Mr Western's conclusions. (K.S.)

Table I

The incidence of smut on samples of the experimental material grown to maturity

Ref. no. of smut	Host	No. healthy	No. smutted	Smutted plants (%)
L_1	Strigosa	39	0	0
L_1	Potato	2	35	95
L_{11}	Strigosa	0	33	100
L_{11}	Potato	40	0	0
C_1	Markton	38	0	0
C_2	Markton	37	0	0
C_4	Markton	38	0	0
L_1	Markton	38	0	0
L_2	Markton	40	0	0
L_{11}	Markton	38	0	0

Text-figs. 1 and 2 were made by drawing a median section with the aid of the camera-lucida and including all the mycelium visible with the low power of the microscope in five sections, *i.e.* the median section and the two on either side of it.

III. Presentation of results

(1) *The invasion of susceptible oats.* Ustilago Avenae *form L_1 on* Avena sativa *variety Potato* (2855) *and* Ustilago Avenae *form L_{11} on* Avena strigosa (521)

Seedlings of the varieties Potato and Strigosa, infected with smuts to which they were completely susceptible were fixed and examined at 7 and 21 days. As the development of the pathogen within the host was similar in both cases they may conveniently be described together and will serve to illustrate the earlier stages in a course of events which ultimately leads to the production of smutted panicles.

With one exception all susceptible plants examined at 7 days contained abundant mycelium in the coleoptile and mesocotyl, but none in the young growing points themselves. The individual hyphae appeared to be growing consistently towards the young meristematic regions of the seedling and were mostly intracellular at this stage, but occasional pieces of intercellular mycelium were present in the more deeply seated host tissues. It was quite usual to find long hyphal strands growing in close association with the phloem elements of the stele of the mesocotyl and coleoptile (Plate VII, fig. 2). In no instance was mycelium found crossing the gap between the coleoptile and the first true leaf. In older cells which had become vacuolated the individual hyphae were often found to be devoid of contents. Such empty portions of mycelium were common in both resistant and susceptible varieties.

A study of the parasitisation of the susceptible variety Victor by *Ustilago Avenae* has been made by Kolk (6), in which she describes the association of hyphae and host cytoplasm. This relationship was evident in the varieties examined in the present work, for the host cytoplasm was usually found to be intimately associated with a hypha passing through the cell. There was no indication that either the host or pathogen suffered from this contact but, on the contrary, both appeared to be normal and the mycelium grew rapidly. In susceptible seedlings 3 weeks old, the young growing points were always found to be heavily infected with mycelium consisting of numerous, short, thickened and branched hyphae which appeared to be lying among the cells rather than actually penetrating the walls (Plate VII, fig. 1).

(2) *The invasion of resistant oats.* Ustilago Avenae *form L_1 on* Avena strigosa (521) *and* Ustilago Avenae *form L_{11} on* Avena sativa *variety Potato* (2855)

The examination of the resistant hosts was made on seedlings fixed after 3, 7, 14 and 21 days. In both cases selected for study, the smuts succeeded in penetrating the epidermis of the host and in giving rise to mycelium which developed rapidly, particularly in the parenchyma of the coleoptile. Successful penetrations were, however, definitely more abundant in the case of strain L_1 parasitising Strigosa, and the resulting mycelium was more plentiful.

As far as it was possible to ascertain, the infection hyphae appeared to be produced from the germinating spores directly and not from sporidia. Several chlamydospores were observed in which the promycelium, or an equivalent structure, was definitely penetrating the cell wall. Penetration is shown in Text-fig. 3, but in this example sectioning has removed the spore. Once inside the epidermal cell wall, the entering infection hypha usually swells to form a funnel-shaped vesicle, which, while still living, does not take up the violet stain. No response of the host cells to the entry of hyphae could be detected in these two cases. The swollen portion tapers, giving rise to an ordinary mycelial strand which traverses the coleoptile at right angles and penetrates the cell walls as it encounters them. At these points the hyphae are swollen (Plate VII, fig. 4). After passing through two or three cells in this manner, the hyphae usually change their direction and grow parallel with the long axis of the coleoptile. The fact that quite long pieces of mycelium were found in seedlings 3 days old suggests that penetration must have been effected only a very short time after the grains were planted.

The examination of the older resistant seedlings proved particularly interesting, since it soon became apparent from the 7-day material that form L_{11} on Potato was unable to develop to the same extent as L_1 on Strigosa, even though both of these hosts appear completely resistant to these particular strains of smut when the absence of smutted panicles at maturity is taken as the criterion.

The invasion of Potato by strain L_{11} will be described first. Seedlings of this variety a week old showed numerous empty hyphae and sometimes a discoloration of host cells. Normally the presence of smut mycelium appears to have no ill effects upon the host cell containing it. This was noticed by Lutman(7) and Kolk(6), both of whom studied susceptible varieties. In the case of the resistant varieties now under discussion there were indications that this does not always hold. In addition to the empty and apparently dead hyphae already mentioned, many swollen portions of mycelium were present, often closely surrounded by layers of the host cytoplasm. This enlarged and degenerated mycelium appeared to be ultimately ingested by the host cells.

Material fixed at 14 days and later was almost devoid of mycelium, traces of degenerated hyphae occurring in the coleoptile and mesocotyl only. It seems that very little mycelium of strain L_{11} develops an intercellular habit of growth on the variety Potato.

Strain L_1 parasitising Strigosa (521) differed from L_{11} upon Potato in certain particulars. It was apparently able to persist and develop to a much greater extent. In seedlings sectioned after 7 days, intracellular mycelium which appeared to be quite healthy was found to be plentifully distributed throughout the coleoptile and mesocotyl. In sharp contrast to plants of Potato inoculated with strain L_{11}, all of the 14 days old seedlings of Strigosa infected with strain L_1 were found to contain mycelium. This was both intra- and intercellular in nature and was for the most part associated with the stelar tissue (Plate VII, fig. 5). Occasionally hyphae were present in the base of the node (Text-fig. 1) but not near the young growing point itself, access to which appeared to be prevented by a compact block of regularly shaped cells, extending in an arc formation and effectively cutting off the young leaves and growing point from the tissues immediately below them. In no case was mycelium observed among these cells. The most healthy-looking hyphae growing towards the node have been photographed and are shown in Plate VII, fig. 3. It will be noticed that they are growing in close association with the stelar tissue of the mesocotyl and appear to be quite vigorous.

In no case was the growing point of a 21-day plant invaded by mycelium, and that present in the mesocotyl and coleoptile showed signs of degeneration. Much of it was swollen, usually more or less branched, and chiefly confined to the stelar tissues of the mesocotyl (Text-fig. 2).

(3) *The invasion of Markton by* Ustilago Avenae (*forms L_1, L_2, L_{11}*) *and* U. Kolleri (*forms C_1, C_2, C_4*)

Markton, a variety of *Avena sativa*, has always proved to be highly resistant, and at Aberystwyth has never produced smutted panicles when inoculated with any of the biological species of smut under discussion. In spite of this apparent immunity, mycelium succeeds in penetrating the young seedlings and may persist for some time in certain regions of the host plant.

A microscopical examination of the early stages of the penetration and invasion of this host by six collections of smut has been made and the results are recorded below.

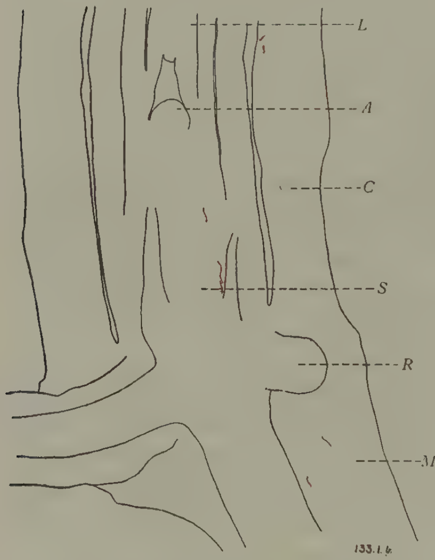
In this experiment three strains of loose smut, numbered L_1, L_2,[1] and L_{11}, and three strains of covered smut, C_1, C_2, and C_4 were used.

The results are of distinct interest in showing that the resistance offered by Markton to these smut collections is of a different order from that already described in the other resistant varieties invaded by the same biological species of smut.

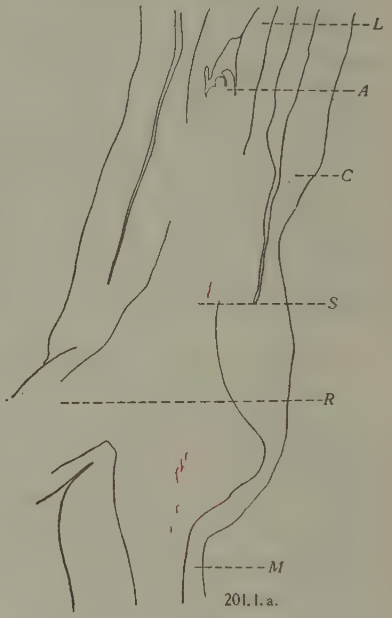
It at once became clear that the host reaction to the different smuts was certainly not uniform. In the case of forms C_1 and L_{11}, the spores germinated and gave rise to germ tubes which succeeded in penetrating the cuticle of the prospective host cell, but failed to make their way into the cell cavity. This was due, apparently, to a host response resulting in

[1] See footnote, p. 247.

the deposition of a pad of material which successfully prevented the entry of the infection hypha (Text-fig. 3, Plate VIII, figs. 1 and 2). A more detailed description of this structure is given below in the section dealing with the response of the host (p. 254). That this failure to cause infection is not due to any lack of energy on the part of forms C_1 or L_{11} is shown by the fact that they are able to penetrate and produce abundant mycelium on congenial hosts, even in seedlings 3 days of age (Text-fig. 5).



Text-fig. 1



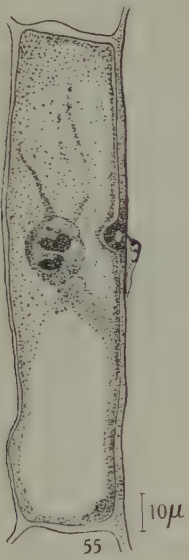
Text-fig. 2

Text-fig. 1. Median section of a seedling of *Avena strigosa* (521), 14 days old, invaded by mycelium of *Ustilago Avenae*, form L_1.

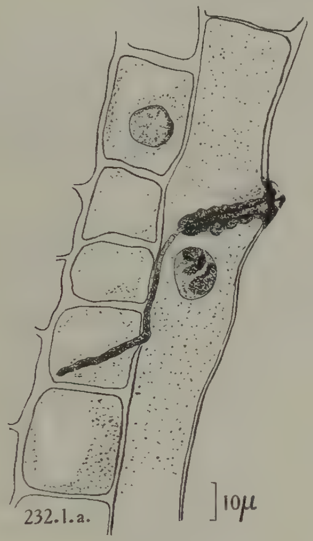
Text-fig. 2. The same variety and smut but in a seedling 3 weeks old. The mycelium has made no further progress and the growing point remains uninfected. *A* = growing point. *C* = coleoptile. *L* = true leaf. *M* = mesocotyl. *S* = stele. *R* = root. Mycelium shown in red.

The strain of *Ustilago Kolleri*, C_4, differed from those already described in that although a host response was again evident, it took a rather different form, resulting in the formation of a sheath-like structure which did not, however, prevent the ultimate penetration of the cell by an infection hypha (Text-fig. 4, Plate VIII, fig. 4). The nature of this sheath and its development are discussed on p. 255. The germ tubes which were successful in escaping from the sheath gave rise to a certain amount of mycelium which could be found in seedlings 3 days and 1 week old, but never at any later stage.

The two collections of *Ustilago Avenae*, L_1 and L_2, gave similar reactions on Markton and may be described together. Both succeeded in penetrating the host epidermis and no host response could be detected at this stage. The infection tubes were of a uniform width and stained normally with gentian violet. There was no well-defined vesicle inside the epidermal wall in either of these strains. Hyphae were present in seedlings examined after 7 days, but many of these were degenerated and the host cells were often markedly distorted and deeply stained. No mycelium was found to persist beyond 7 days.



Text-fig. 3



Text-fig. 4

Text-fig. 3. Attempted penetration of the epidermal cell of a Markton seedling 3 days old, showing the thickening of the cell wall and the thin peg-like tip of the germ tube.

Text-fig. 4. Sheath surrounding the penetrating germ tube of the form C_4. In this case the hypha escapes from the sheath and passes to the inner tissues of the host.

The course of events in the attack of strain C_2 upon this host proved to vary considerably from that in all the other infections investigated. Initial penetration was characteristic (Plate VIII, fig. 5), and a well-defined vesicle was easily seen immediately inside the cell wall. The hypha to which this gave rise was usually well stained and no host response could be detected. The mycelium seemed to progress easily through the cells of the coleoptiles of seedlings 3 days old in a manner which recalled the invasion of Potato by strain L_{11} (p. 250). In plants a week old, mycelium could be found growing up and down the coleoptile, in addition to some which penetrated the parenchyma cells at right angles to the long axis. The host response in this case was not marked.

In material fixed at 14 days apparently healthy mycelium was still found, but only in cells bordering the stele of the coleoptile (Plate VII, fig. 6). The individual hyphae, which were thickened and often branched, were especially common among the phloem elements and were mostly intracellular (Text-fig. 6).

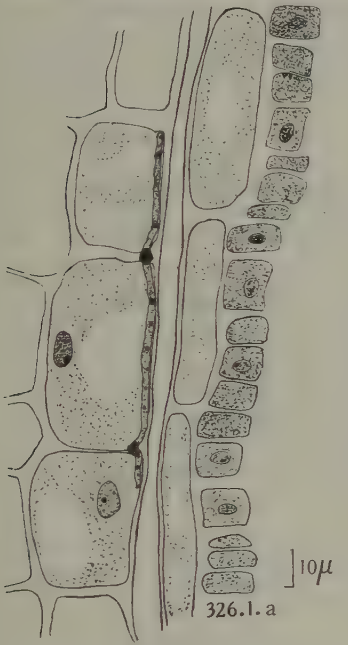
In plants 3 weeks old, traces of mycelium could be found in the phloem of the coleoptile, but this structure was, at this stage, becoming disorganised. No other strain of smut studied proved to be capable of surviving as long as C_2 on Markton, and, even here, the only mycelium persisting for any length of time was that found closely associated with the stele of the coleoptile.

Taking the results as a whole, it may be said that Markton is extremely resistant to invasion by all of the smuts concerned, and that mycelium, even in the most advanced cases, is only able to survive for an appreciable time in the tissues of the coleoptile or the mesocotyl. It was never found in the base of the node or in any of the young leaf tissues. In this respect Markton resembles the other resistant varieties already described, but it is so far unique in that its epidermal wall offers an impassable barrier to at least two forms of smut.

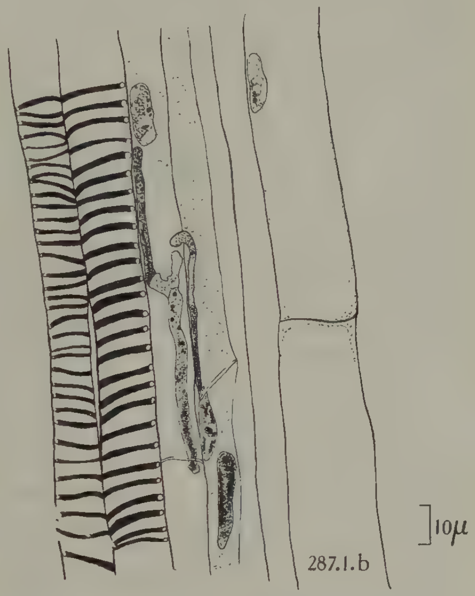
(4) *The response of the host*

It has already been stated that the response of the cell to invasion by smut hyphae varied according to the identity of the host and pathogen involved. The best example of this differential response to distinct strains of smut is provided by the variety Markton. Here, with forms C_1 and L_{11}, the cell wall gives rise to a pad of material which completely prevents the entry of the germ tube. In order to determine the nature of this deposit, microchemical tests were undertaken, the results of which are recorded in Table II. Reactions given by the pad of material resembled closely those of the inner layer of the epidermal wall, while the observations made on various stages in its development suggest that it is in direct continuity with the cell wall (Text-figs. 3, Plate VIII, fig. 1 and 2). Frequently it was possible to observe in the cuticle a hole with a swollen margin, the position of which, when seen in section, was marked by two spots which stained red with Sudan III. The reaction with chlorzinc-iodide, azo-blue and iodine followed by sulphuric acid suggested that the pad itself is related to cellulose.

The action of strain C_4 was rather different, in that although a deposit was laid down as before, it was of a different shape and did not react in quite the same way to microchemical tests. An early stage of penetration is figured in Plate VIII, fig. 3, which shows the hole in the cuticle and the effect on the epidermal cell wall. Immediately inside the wall is seen the entering tube swollen to form a pear-shaped structure with a peg-like outgrowth at its distal end. The host cytoplasm is invaginated and the plasma membrane and the limiting wall of the germ tube appear, in the actual preparation, as a double structure with a brownish deposit between them. It is apparently this material which increases to form a sheath-like structure, often having an undulating margin and investing the still visible infection tube (Plate VIII, fig. 4). The formation of this structure apparently proceeds along similar lines to the development of the haustoria in the Erysiphaceae described by Smith (16). Fellows (5)



Text-fig. 5



Text-fig. 6

Text-fig. 5. An intracellular hypha of form C_1 on Strigosa in a seedling 3 days old. It has already crossed the coleoptile and is now progressing up the inner epidermis.

Text-fig. 6. Characteristic thickened mycelium of form C_2 associated with the phloem and xylem of the coleoptile of a Markton seedling 14 days old.

has figured protuberances in the cells of wheat invaded by *Ophiobolus graminis,* to which he has given the name lignitubers. Microchemical tests have failed however to reveal the presence of lignin in the present instance; neither does it appear that the ingrowths are purely cellulose, as they do not always assume a blue colour with chlorzinc-iodide. They dissolve slowly in zinc chloride solution, but hot 3 per cent. sulphuric acid fails to affect them.

Grant Smith observed that the material composing the distal end of the haustorial sheaths was of a slightly different nature from that of the proximal end in connection with the host cell wall. He noticed a difference in their reaction to stains and assumed that the distal portion of the sheath undergoes some modification preparatory to the emergence of the haustorium. Similar changes may occur in this case and be responsible for the occasional differential staining of the cell wall and the protuberance to which it gives rise.

Table II

Microchemical tests upon the sheaths and deposits formed by Markton in response to initial penetration by Ustilago Kolleri, *forms C_1 and C_4*

Reagent	Cuticle	Epidermal wall	Deposit or sheath
Chlorzinc-iodide	Unstained	Blue grey	C_1 blue grey C_4 grey-brown
Iodine	Unstained	Yellow	Yellow
Iodine followed by sulphuric acid	Unstained	C_1 Yellow changing to grey blue C_4 Grey blue after some time	
Azo-blue	Unstained	Blue	C_1 blue C_4 dull blue
Zinc chloride solution in hydrochloric acid	No action	Dissolved slowly	Contracted and dissolved slowly
20% solution of potassium hydroxide	No action	Swollen	Swollen and in C_4 partially dissolved
Sudan III in glycerine and alcohol	Red	Unstained	Unstained
Phloroglucin and hydrochloric acid	Unstained	Unstained	Unstained
Aniline chloride	Unstained	Unstained	Unstained
Methylene blue	Unstained	Blue	Blue
Hot 3% solution of sulphuric acid	No action	No action	No action

Similar structures have been figured by Brefeld (3) and Wolff (21) in the smut fungi, while, in addition to the work of Grant Smith already quoted, Allen (1) and Rice (10) have described a closely related type of behaviour in the penetration of the host cell by the haustoria of certain strains of wheat-stem rust. These sheath formations were not found in the less resistant varieties studied, but occasionally the empty portion of a hypha which had grown well into the host tissues could be traced to its original point of entry, and the remains of the thin-walled primary vesicle seen as a rigid funnel-shaped structure which stained with gentian violet (Plate VII, fig. 4).

Walter (18), in a recent paper upon the mode of entrance of *Ustilago zeae* into maize, observed that in many cases the germ tubes seemed to be attracted to the nuclei of the host cells, and Allen (2) has recorded a similar relationship between the haustoria of *Paucinia graminis* and the nuclei of the host cells. No indications that this is true in the case of the oat smuts have been obtained in the present work, the germ tubes and the nuclei of the epidermal cells appearing to be quite indifferent to the presence of each other. With older and degenerating mycelium, on the other hand, it is not uncommon to find the nuclei of several adjacent host cells in intimate association with a strand of mycelium (Plate VII, fig. 4), but it is not easy to say if this has any bearing upon the problem of resistance, or if it is merely a natural consequence of the maturing of the cell and the ensuing contraction of the cytoplasm around the walls and the included hyphae.

The occurrence of sheath-like formations other than those associated with penetrating primary infection tubes have been recorded on several occasions in the smut fungi. Rice (10) has listed some of the papers which contain references to them. Several examples of hyphae surrounded by layers of cytoplasm have been noticed in the present work, but their exact significance, if any, is difficult to determine since they are not confined exclusively to a particular host-pathogen complex. All intracellular mycelium appears to follow the distribution of the cell cytoplasm very closely, without, as a rule, showing any sign of disharmony with it. As the hyphae become more mature, however, and almost devoid of contents, cytoplasm tends to accumulate around them, while still older degenerating strands appear to be gradually ingested by the host cell and finally disappear. This happens more frequently in resistant varieties, but it is not confined exclusively to them. Sheathing of these hyphae appears to be a secondary effect rather than the actual cause of their degeneration, and it is probably those portions of mycelium which, for some reason, are already in an unhealthy state, which are particularly liable to present this appearance. If this is so it seems natural that these sheaths occur more abundantly in resistant varieties.

It is well known that certain fungi invading host tissues secrete substances which penetrate the cells in advance of the mycelium and bring about their disorganisation or death (Marshall Ward (20), Allen (1), Walter (18)). The question arises whether some such substance may be involved in the case of parasitism by the oat smuts. In a detailed histological study of the host-pathogen relationship in *Ustilago Avenae*, Wang (19) found that occasionally the cell nuclei in advance of the invading hyphae may undergo some alteration before the other cell constituents show any modification and before the actual penetration of the cells concerned has taken place. Some evidence of "action in advance" has been obtained in the present investigation in Markton invaded by forms L_1, L_2 and C_4, and to a very slight extent in the older tissues of Potato parasitised by form L_{11}.

Thus in seedlings of Markton, 7 days old, containing mycelium of the strains L_1, L_2, and C_4, areas of host tissue associated with the fungus appeared to have undergone considerable disorganisation. In such necrotic areas the cell walls were distinctly swollen, the protoplasm contracted, while both the walls and the remains of the protoplasm stained heavily with gentian violet. Frequently these cells were in advance of the parasite and had not suffered penetration by it. The fungus also showed signs of marked degeneration and the individual hyphae stained deeply (Plate VIII, fig. 6). Necrosis of this type suggests a parallel with the condition found in certain hypersensitive wheats attacked by stem rust (Stakman (17), Allen (1)), but it is not sufficiently general in its occurrence for one to conclude that hypersensitiveness alone may explain the phenomenon of smut resistance in oats. In sharp contrast to the examples quoted there are other resistant oats (*e.g.* Strigosa invaded by form L_1) in which mycelium can persist for a comparatively long time with no visible expression of incompatibility in the cells of either host or pathogen.

IV. DISCUSSION

A histological study of seedlings grown from smutted grain has shown that varieties of oats differ widely in their powers of resistance to attack by smut fungi. Since these differences in behaviour are remarkably constant, it has been possible to classify the oat varieties studied into definite grades of resistance which are listed, with their distinguishing characteristics, in Table III.

A resistance almost amounting to complete immunity has been met with in the case of Markton inoculated with the biological species C_1 and L_{11}, where the infection tube fails to pass beyond the epidermal cell wall

(Grade 1). On the other hand, the resistance offered may be so slight that the plants produce only smutted panicles at maturity (Grade 5). Intermediate between these two extremes, three further types occur, the characteristic features of which may be summarised as follows:

In the case of forms C_4, L_1, and L_2 parasitising the resistant variety Markton, penetration of the host is successful but the development of internal mycelium is very slight and, in seedlings 7 days old, it is accompanied by marked necrosis in the host tissues (Grade 2).

Table III

Grades of resistance shown by certain varieties of oats to biological species of smut

	Host-pathogen relationship at				
Grade	3 days	7 days	14 days	21 days	Examples
1	Penetration fails	—	—	—	C_1, L_{11}, on Markton
2	Penetration successful. Mycelium in coleoptile	Mycelium in superficial tissues only. Some necrosis of host cells	—	—	C_4, *L_1, L_2, on Markton
3	Penetration successful. Mycelium in coleoptile	Intracellular mycelium in coleoptile and mesocotyl	Mycelium mostly degenerated but some restricted to stele of coleoptile	Entirely absent except for isolated traces in coleoptile	C_2 on Markton, L_{11} on Potato
4	Penetration successful. Mycelium abundant	Intracellular mycelium in coleoptile and mesocotyl	Mycelium both intracellular and intercellular and in deeper tissues	Remains of mycelium in mesocotyl but growing point and meristems uninfected	L_1 on Strigosa, C_4 on Strigosa†
5	As 4 above	As 4 above	—	Mycelium very abundant. Growing points and meristems heavily infected	L_1 on Potato, L_{11} on Strigosa

* See footnote, p. 247. † Sampson (15).

A slightly less resistant type is recognised in which the smut persists within the host for a longer period with only slight necrosis of the host cells. The smut forms, C_2 on Markton and L_{11} on Potato, provide examples of this grade (Grade 3).

Still less resistance is shown by those varieties in which the smut mycelium persists in an apparently healthy condition for a considerable period, but fails to reach the young meristematic tissues at the growing points. The forms L_1 and C_4 on Strigosa are good examples of this group (Grade 4).

An examination of these grades which would doubtless be multiplied by further study shows that the "mechanism of resistance" developed by oat varieties in response to attack by smut fungi is not always expressed in the same way. In the first place it may be due to a failure to penetrate the epidermal cell of the host. This has been found in the case of Markton inoculated with spores of the forms C_1 and L_{11}. It is of interest that both these collections, which induce an identical response in the hexaploid variety of *Avena sativa*, Markton, normally parasitise the diploid oats, *Avena strigosa* and *A. brevis*. The second defence mechanism has also been found in Markton and takes the form of a hypersensitive reaction, somewhat similar to that in wheat rust, which results in the rapid degeneration of the smut mycelium together with considerable necrosis of the host cells. The third and apparently most common expression of incompatibility between oat varieties and smut is a restriction of the growth rate of the mycelium which allows the growing points to escape infection. This type of reaction was recognised in certain smuts by Brefeld (3) and Sampson (15) and in resistant wheats by Woolman (22). It has been shown by the writer that the depressant action does not always operate at the same growth stage or to the same extent in different combinations of host and pathogen strains, a fact which has been made use of in constructing the table showing grades of resistance (p. 259).

It is not known if the biochemical processes underlying the three ways in which resistance finds expression have anything in common or if they are quite unrelated, but it is of interest to find them operating in one single disease, yet characteristic of particular combinations of variety and strain in host and pathogen. The work emphasises the need for a critical regard to purity of material in any study of parasitic relationships.

V. SUMMARY

1. The progress of the mycelium of two forms of *Ustilago Avenae* within resistant and susceptible hosts has been described and attention drawn to differences between them.

2. Form L_1 on *Avena strigosa* was found to persist longer than form L_{11} on the variety Potato, although both of these oats failed to produce infected panicles with these smuts.

3. The response of the resistant variety, Markton, to attack by six collections of *Ustilago Avenae* and *U. Kolleri* was found to vary considerably. Forms C_1 and L_{11} failed to penetrate, while form C_2 entered easily and the mycelium persisted for some time.

4. The types of sheathing structures around the penetrating tubes have been found in Markton inoculated with forms C_1 and C_4. Their reaction to microchemical tests was investigated.

5. A hypersensitive reaction resulting in the formation of necrotic areas around the parasite hyphae has been found in Markton invaded by forms C_4, L_1 and L_2.

6. The varieties studied have been classified according to the grade of resistance offered to particular forms of smut. Five grades have been described.

7. In oats, resistance to smut is expressed in at least three ways, (i) a reaction of the epidermal cell wall which prevents penetration, (ii) necrosis of host cells, and (iii) a retarding effect on the growth of mycelium within the host.

VI. Acknowledgments

The writer wishes to express his sincere thanks and to acknowledge his indebtedness to Miss K. Sampson, M.Sc., for the provision of material, for permission to include the present work in her series of papers on the "Biology of oat smuts", and especially for her constant interest and advice during the progress of the work.

Grateful acknowledgment for the provision of excellent working facilities is made to Prof. R. G. Stapledon, C.B.E., M.A., Director of the Welsh Plant Breeding Station.

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EXPLANATION OF PLATES VII AND VIII

PLATE VII

Fig. 1. 33.1. *a* (L_{11} on 521). Short pieces of intercellular mycelium in the growing point of a susceptible oat seedling 21 days old. ×97.

Fig. 2. 29.2. *b* (L_1 on 2855). Long hypha with empty portion growing along the stele of the coleoptile of a susceptible seedling 21 days old. ×97.

Fig. 3. 133.1. *b* (L_1 on 521). Mycelium associated with the stele in the base of the node of an oat seedling 14 days old, belonging to a variety which shows mature resistance. ×97.

Fig. 4. 131.1. *b* (L_1 on 521). Funnel-shaped structure and degenerated piece of intracellular mycelium in a resistant seedling 14 days old. ×105.

Fig. 5. 132.1. *b* (L_1 on 521). Thickened, branched mycelium in the stele of the mesocotyl of an oat which shows mature resistance. This type of mycelium is characteristic of older seedlings of resistant varieties Grade IV. 14 days. ×97.

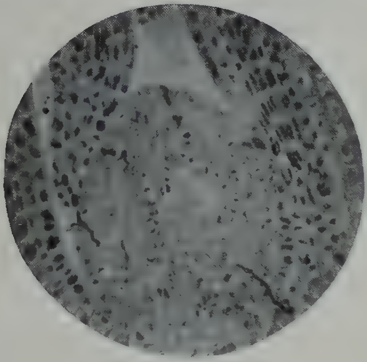
Fig. 6. 287.1. *b* (C_2 on Markton). An apparently healthy hypha growing along the stele of the coleoptile of a seedling 14 days old of the very resistant variety Markton. ×105.

PLATE VIII

Fig. 1. 221.1. *b* (C_1 on Markton). Seedling 3 days old. The entering germ tube has been cut through. A well defined deposit has been laid down by the host cell which effectively prevents further development. ×612.

Fig. 2. 248.1. *a* (L_{11} on Markton). Seedling 3 days old. Similar to above. Note the hole in the cuticle at the point of entry. ×612.

Fig. 3. 343.1. *b* (C_4 on Markton). Seedling 2 days old showing early stage of penetration and beginning of sheath formation. The host cytoplasm is invaginated. ×612.



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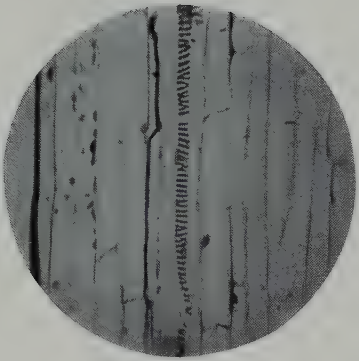

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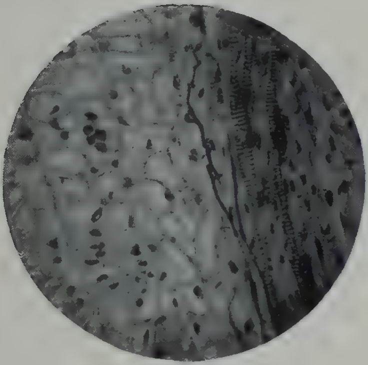

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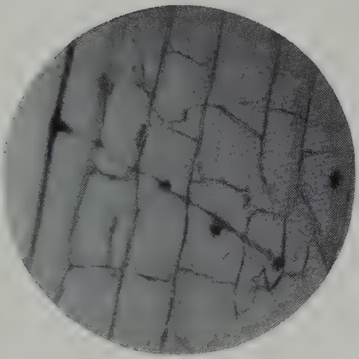

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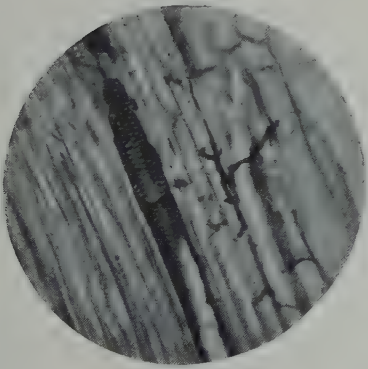

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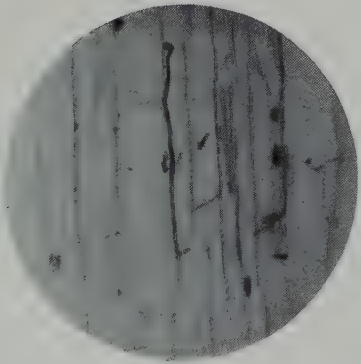
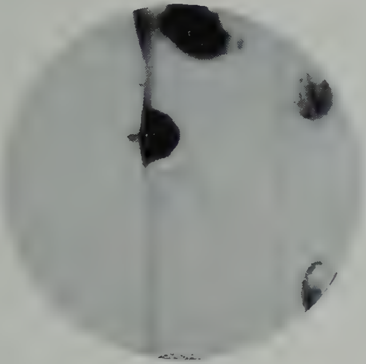


Fig. 6.

WESTERN.—The Biology of Oat Smuts (pp. 245–263).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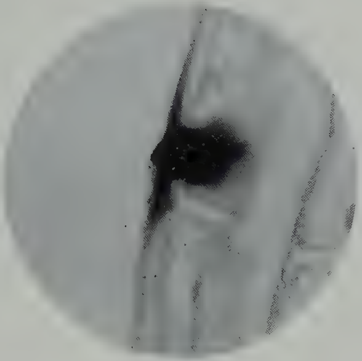
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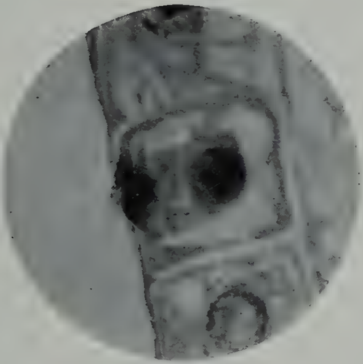
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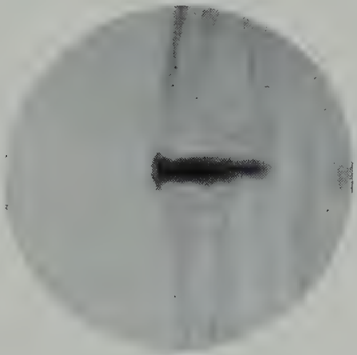
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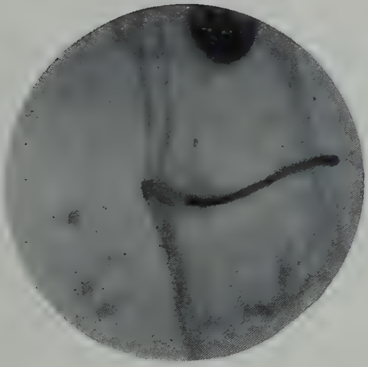
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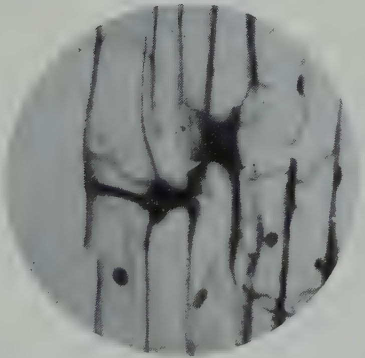
Fig. 6.

WESTERN.—The Biology of Oat Smuts (pp. 245–263).

Fig. 4. 232.1. *a* (C_4 on Markton). Seedling 3 days old. The penetrating tube is invested by a sheath formed by the host but in this case it does not completely prevent the entry of the pathogen. ×612.

Fig. 5. 57.1. *b* (C_2 on Markton). Penetration of the infection hypha. Note the hole in the cuticle seen in side view and the empty swollen vesicle immediately within the epidermal cell wall. Seedling 3 days old. ×612.

Fig. 6. 267.1. *a* (L_1 on Markton). Penetrating hypha which has failed to progress beyond one or two cells. Note the disorganised and deeply stained host cells (7-days seedling). ×612.

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A TEMPERATURE STUDY OF *PYTHIUM* ATTACK ON SWEDE SEEDLINGS

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(With Plate IX)

Introduction

In the course of raising swede seedlings in pots filled with unsterilised soil it was observed that considerable variation occurred in the seedling stands produced in different pots although the same number of seeds had been sown in each pot. Thus, pots which had been prepared in exactly the same way and which had received the same treatment often exhibited differences of more than 30 per cent. in the numbers of seedlings established. On examining a pot showing a poor stand it was found that in many cases the seed had commenced to germinate but that the primary root had been killed soon after its extrusion from the seed coat. In other cases the death of the seedling had occurred somewhat later but before it had been able to reach soil level. From one of the diseased seedlings a pure culture of a species of *Pythium* belonging to the *P. de Baryanum*[1] group was isolated. Subsequent work carried out to test the pathogenicity of this fungus showed that it was strongly parasitic and responsible for the poor stand of seedlings by setting up a pre-emergent phase of seedling disease. Typical damping-off had not been observed during the course of the work.

It was decided to make a further study of the disease, and the present investigation was undertaken with the object of obtaining information upon the two following points:

(*a*) The effect of the temperature under which the seedlings are raised upon the incidence of the pre-emergent and damping-off phases of the disease.

(*b*) Whether the disease may be prevented by disinfecting the seeds with an organic mercury compound prior to sowing.

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[1] The writers' thanks are due to Mr S. F. Ashby, of the Imperial Mycological Institute, who has examined the fungus and suggests that although it is not true to type, it should be placed in the *Pythium de Baryanum* group.

THE EXPERIMENT

The two varieties of swede chosen for this work were Ideal and Tipperary, and the seed samples used gave on analysis a high percentage of purity and germination. Six-inch flower pots were used, one hundred seeds being sown in each pot. Each pot was placed in a saucer which was kept filled with water whereby the soil was kept uniformly moist throughout the experiment. The soil was sterilised before use by autoclaving the filled pots with their saucers for 20 min. at 15 lb. pressure. The inoculation of the soil and the sowing of the seed was carried out after the sterilised soil had been rested for 7 days.

The fungus to be used for inoculation purposes was grown on plates of 2 per cent. malt extract agar, and at the time of inoculation the cultures had just reached the edges of the plates. Two plates were used for inoculating each pot, the contents of one plate being incorporated in the soil before the seed was sown and those of the second in the covering soil. There were therefore present layers of inoculum above and below the seed. The soil of each control pot was treated in a similar manner with the contents of two uninoculated plates of 2 per cent. malt extract agar. The seed which was to be disinfected was dusted with a proprietary organic mercury preparation known to be effective in the prevention of certain seed-borne diseases of cereals.

Three similar series of pots were prepared, each series consisting of sixteen pots made up as follows:

(1) Variety Tipperary:

Two pots with non-inoculated soil, sown with non-disinfected seed.
Two pots with soil inoculated with *Pythium*, sown with non-disinfected seed.
Two pots with non-inoculated soil, sown with disinfected seed.
Two pots with soil inoculated with *Pythium*, sown with disinfected seed.

(2) Variety Ideal:

Eight pots set up as for the variety Tipperary.

Immediately after preparation each of the three series of sixteen pots was placed under conditions where the only variable growth factor was that of temperature. The *high-temperature series* was placed in a heated greenhouse, the *medium-temperature series* in a cool greenhouse, while the *low-temperature series* was kept out of doors during the day and, as the work was carried out in the months of February and March, the pots were brought into a cool greenhouse at night in order to avoid severe frosts. A thermograph record was kept for each series throughout the course of the experiment. An analysis of the prevailing temperatures is given in Table I, where the temperature range has been divided into intervals of 5° C., and the number of hours given during which each series was exposed to each interval of temperature.

Table I

Distribution of temperature in hours

Intervals of temperature °C.	High-temperature series hours	Medium-temperature series hours	Low-temperature series hours
0–5	0	0	268
5–10	0	182	511
10–15	0	182	80
15–20	46	13	5
20–25	132	0	0
25–30	80	0	0
Duration of experiment	258	377	864

The average temperature for each series, estimated by recording the temperature at intervals of 2 hours throughout the whole experiment, was as follows:

High-temperature series	23·0° C.
Medium-temperature series	10·2° C.
Low-temperature series	5·8° C.

OBSERVATIONS

Owing to the wide temperature range, and the resultant differences in growth rate shown by the different series, the experimental period varied largely for each series. General emergence of the seedlings above soil-level occurred after a period of 8 days for the *high-temperature series*, 11 days for the *medium-temperature series* and 26 days for the *low-temperature series*. Final observations as to the incidence of disease were made for the *high-temperature series* after 11 days, for the *medium-temperature series* after 16 days, and for the *low-temperature series* after 36 days. Neither the low nor the high conditions of temperature were good for the growth of the seedlings, for whereas in the former case they were stunted, in the latter they were drawn and etiolated.

RESULTS

The results are set out in Table II, where it will be seen at once that seed disinfection with an organic mercury compound failed to control the disease. This being so, the two series in which non-disinfected seed and disinfected seed were used may be regarded as comparable. At the same time it will also be seen that there is no significant difference in disease susceptibility in the case of the swede varieties used. It is therefore permissible to regard these series as comparable. The experiment may

Table II

Results obtained from a temperature study of Pythium *attack on swede seedlings*

Seed treatment	Variety of swede	Soil treatment	Emergent seedlings %	Decrease of emergence due to disease %	Typical "damping-off" %	Total of diseased seedlings %
		High-temperature series				
Non-disinfected	Tipperary	Non-inoculated	78·0	—	—	—
		Inoculated with *Pythium*	69·0	11·5	11·5	23·0
	Ideal	Non-inoculated	78·0	—	—	—
		Inoculated with *Pythium*	64·0	17·9	5·2	23·1
Disinfected	Tipperary	Non-inoculated	77·0	—	—	—
		Inoculated with *Pythium*	71·0	7·8	19·5	27·3
	Ideal	Non-inoculated	85·0	—	—	—
		Inoculated with *Pythium*	71·0	16·5	7·1	23·6
		Medium-temperature series				
Non-disinfected	Tipperary	Non-inoculated	85·0	—	—	—
		Inoculated with *Pythium*	75·0	11·8	0·0	11·8
	Ideal	Non-inoculated	79·0	—	—	—
		Inoculated with *Pythium*	66·0	16·5	0·0	16·5
Disinfected	Tipperary	Non-inoculated	75·0	—	—	—
		Inoculated with *Pythium*	63·0	16·0	0·0	16·0
	Ideal	Non-inoculated	89·0	—	—	—
		Inoculated with *Pythium*	72·0	19·1	0·0	19·1
		Low-temperature series				
Non-disinfected	Tipperary	Non-inoculated	82·0	—	—	—
		Inoculated with *Pythium*	52·0	36·6	0·0	36·6
	Ideal	Non-inoculated	86·0	—	—	—
		Inoculated with *Pythium*	58·0	32·6	0·0	32·6
Disinfected	Tipperary	Non-inoculated	80·0	—	—	—
		Inoculated with *Pythium*	52·0	35·0	0·0	35·0
	Ideal	Non-inoculated	85·0	—	—	—
		Inoculated with *Pythium*	64·0	24·0	0·0	24·0

therefore be regarded as having been carried out with eight pots (800 seeds) in each group in place of two (200 seeds) as originally planned.

The total amount of disease resulting from soil inoculation with *Pythium* will be seen to be remarkably consistent in each of the four groups within each temperature series. In the case of the *high-temperature series* an average of 24·2 per cent. of the seedlings were attacked; for the *medium-temperature series* the average was 15·9 per cent. and for the *low-temperature series* 32·1 per cent.

The relation of the incidence of the pre-emergent phase to the damping-off phase is of particular interest. Whereas the damping-off phase was responsible for the death of 10·8 per cent. of the seedlings in the *high-temperature series* as against 13·4 per cent. killed by the pre-emergent phase, in the *medium-* and *low-temperature series* damping-off did not occur at all, the pre-emergent phase being responsible for the total disease.

DISCUSSION

The depredations of *Pythium* spp. in the seed bed giving rise to typical damping-off symptoms are well known, but the pre-emergence phase of the disease does not appear to be so widely recognised. The importance of this early phase has been emphasised by Horsfall (2) in the case of *P. ultimum* attacking tomato seedlings, where he regards the pre-emergence phase of the disease as one of the primary causes of poor stands of tomato seedlings. *P. de Baryanum* has been associated with poor stands in crops of oats and barley (1), while a species of *Pythium* associated with root rot of dent corn (5) may cause a pre-emergent form of seedling disease at soil temperatures of 12–16° C. under conditions of high soil moisture.

The results of these experiments show that the incidence of the pre-emergence and post-emergence phases of *Pythium* attack on swede seedlings is closely correlated with the temperature under which the seedlings are raised given uniform moist soil conditions. At high temperatures the rapid germination and early emergence of the seedlings above soil-level results in the incidence of the pre-emergence phase being kept at a minimum, but the etiolated and drawn seedlings produced under these conditions are subject to typical damping-off. Under conditions of low temperature the rates of germination and emergence are slow, and in this case the incidence of the pre-emergence phase reaches its maximum. Nevertheless the seedlings which do emerge are healthy and not liable to damping-off although their appearance is somewhat stunted. In the case of seedlings raised under conditions of medium temperature the pre-emergence phase of the attack was not significantly greater than for the high-temperature series, while damping-off did not occur at all. It would therefore appear that germination and emergence had been sufficiently rapid to enable the seedlings to escape the pre-emergence attack and that the sturdy seedlings produced were not susceptible to damping-off.

The problem of controlling *Pythium* attack in the seed bed has been attempted by adopting both soil and seed treatments. Steam sterilisation is eminently satisfactory, but it is expensive and there is always the danger of recontamination. Horsfall successfully controlled the damping-off of tomato seedlings by dusting the seed with copper sulphate (monohydrate) (3) or with cuprous oxide (4) and by steeping it in a solution of copper sulphate (2). The use of organic compounds of mercury as dusts and steeps has been tried out by various workers with somewhat contradictory results. The efficient control of the disease by a dust treatment of the seed would be a popular method, and it was for this reason that experiments were carried out with seed disinfected with an organic mercury compound. Although such treatment in no way impaired the germination capacity of the seed, it proved to be useless in so far as the control of *Pythium* attack from the soil is concerned.

In so far as varietal susceptibility of the swede to the disease is concerned, the two varieties used in these experiments proved to be equally susceptible.

From the results obtained it would appear that *Pythium* attack in infected soils is best evaded by the raising of seedlings under temperature conditions sufficiently high to encourage rapid germination and emergence and at the same time favouring the production of non-etiolated and sturdy plants. It is possible that the best results would be obtained by using a combination of two temperatures; a high temperature for a short period to give impetus to germination followed by lower temperature conditions which would ensure the production of hardy and sturdy seedlings.

SUMMARY

1. The type of seedling disease caused by *Pythium* attack on swede seedlings is closely correlated with the temperature under which the plants are raised. Low temperature conditions favour the occurrence of the pre-emergence phase of disease and produce no damping-off. Medium temperatures also produce no damping-off and result in a big reduction

in the pre-emergence phase. High temperatures show the pre-emergence phase at a minimum but encourage damping-off.

2. The disease in general is likely to be best evaded by germinating the seed under conditions of high temperature until the emergence of the seedlings. The temperature should then be lowered in order to encourage the production of sturdy seedlings not likely to be subject to damping-off.

3. In these experiments the disinfection of the seed with an organic mercury compound prior to sowing did not secure any control of the disease.

4. The two varieties of swede used—Tipperary and Ideal—proved to be equally susceptible to the disease.

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EXPLANATION OF PLATE IX

Fig. 1. Swede seedlings raised at a high average temperature. Note the typical damping-off of the seedlings in the inoculated soil.

Fig. 2. Swede seedlings raised at a medium average temperature.

Fig. 3. Swede seedlings raised at a low average temperature. Note the poor stand of seedlings in the inoculated soil.

(*Received October* 25, 1935)

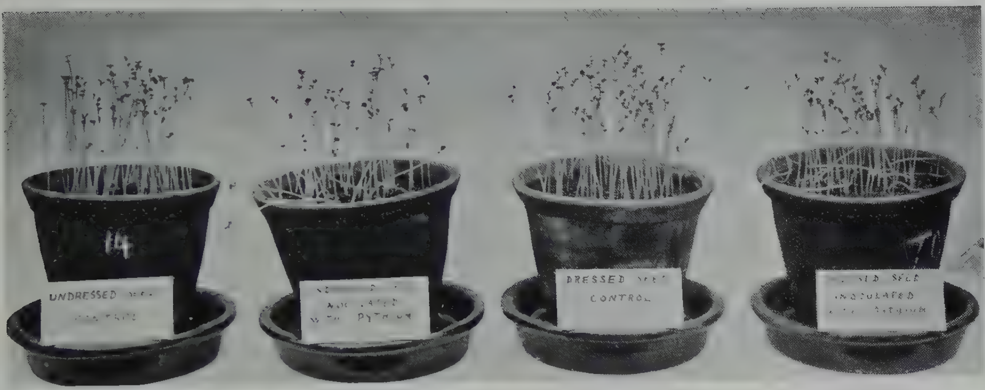
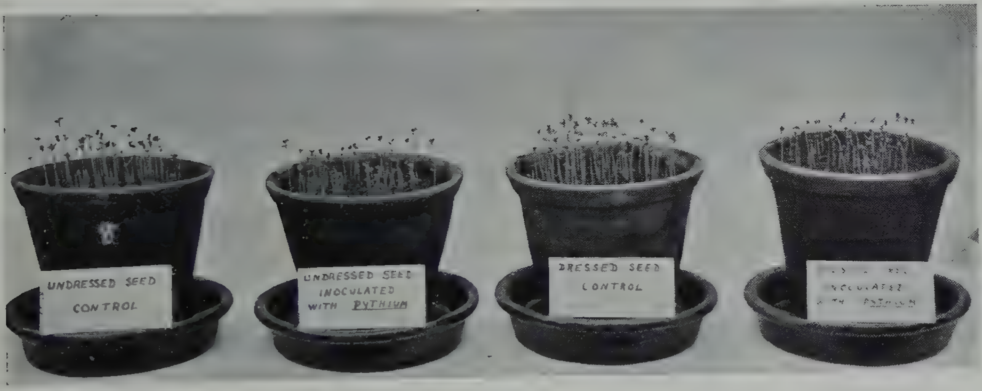

14
UNDRESSED
INOCULATED
WITH PYTHIUM
DRESSED SEED
CONTROL
INOCULATED
PYTHIUM
70

Fig. 1.


UNDRESSED SEED
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UNDRESSED SEED
INOCULATED
WITH PYTHIUM
DRESSED SEED
CONTROL
INOCULATED
PYTHIUM

Fig. 2.

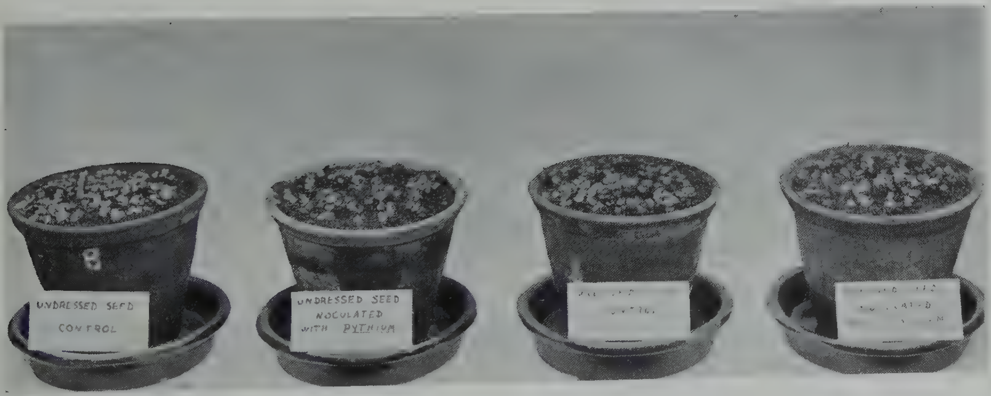
8
UNDRESSED SEED
CONTROL
UNDRESSED SEED
INOCULATED
WITH PYTHIUM

Fig. 3.

GREEVES AND MUSKETT.—A Temperature Study of *Pythium* Attack on Swede Seedlings (pp. 264–270).

THE DETERMINATION OF PHYSIOLOGIC FORMS OF *PUCCINIA TRITICINA* ERIKSS. IN ENGLAND AND WALES

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Little work has previously been done concerning physiologic specialisation within the different species of cereal rusts in this country. An attempt has been made, therefore, to bring the knowledge of specialisation in *Puccinia triticina* into line with that existing in North America and on the continent of Europe.

Historical survey

An account of all the available data on the physiologic forms of *P. triticina* both in America and on the continent of Europe was published by Johnston and Mains (8). The history, therefore, up to that date will be dealt with briefly.

Mains and Jackson (9) described the reactions of the first twelve forms of *P. triticina* on eleven differential wheat varieties. The next three new forms were described by Scheibe (16), who also recorded Form 11 from Central Europe, first described in the United States.

Waterhouse (22) in Australia described two new forms of *P. triticina* which, however, gave identical reactions on the eleven differential wheats and so, according to these reactions, constituted a single form, the number 26 being assigned to it later by Johnston and Mains (8). Waterhouse distinguished them by means of the Australian wheat variety Thew, and designated them "Aust. I" and "Aust. II".

Scheibe (17) described eight new forms from *P. triticina* collected in Germany, Latvia, Esthonia, Poland, Bulgaria, and Hungary. This brought the number of known physiologic forms up to 23 (omitting the new form described by Waterhouse in Australia).

Wellensiek (23), from collections of *P. triticina* in the Netherlands, encountered three forms, Nos. 11, 14, and 15, which had previously been reported in Germany by Scheibe.

Dodoff (2) found Forms 15, 17, 19, and 21 in collections of *P. triticina* made in Bulgaria and described one new form to which the number 24 was assigned.

Form 25 was described by Tscholakow (21) from Hungary.

An aberrant form described by Johnston (6) in the United States was assigned the number 27.

Johnston and Mains (8) described in all twenty-six new forms of *P. triticina* from the United States, of which Forms 28–50 inclusive were found by them, and Forms 51–53 by Schaal, Stakman and Levine. Johnston and Mains provided a key and a table of the infection types of these fifty-three forms.

Since 1932, Form 54 has been isolated by Radulescu (15) from collections made in Roumania, and Mehta (12) has described a new form in India.[1]

MATERIALS AND METHODS

Differential wheat varieties

The wheat varieties used as differential hosts in this study were obtained from Prof. E. B. Mains and Dr C. O. Johnston. They were the same as those used by Johnston and Mains (8), who reduced the number from eleven to eight. The varieties were: Webster, Hussar, Democrat, Mediterranean, Malakof, Loros, Carina, Brevit.

These varieties were grown on the University Farm, Cambridge, and the stock was thus maintained. Wherever possible, however, the original seed from the United States was used. In the variety Webster germination of the original seed became so poor that it was necessary to use seed from the local harvest.

In the variety Loros the original stock was mixed with a fairly high percentage of seeds which produced plants with very hairy leaves. The mixture in this variety gave no trouble as the hairy plants could easily be distinguished and discarded.

Impurities in the original stock of Mediterranean seed, however, gave rather more trouble. The seedlings were indistinguishable morphologically from one another, but there appeared to be a mixture of three varieties. A pot of seedlings, for example, inoculated with a certain physiologic form would often show infection types 4, 2 and 1 on different plants. The reaction of Mediterranean to such a form, therefore, could only be determined after several successive inoculations when a large number of seedlings had given a consistent majority of one type of reaction. The mixture in Mediterranean, however, is not revealed by all physiologic forms of *P. triticina*. In experiments with Form 15, for instance, no reaction other than type 4 has ever been obtained. Scheibe (16) stated that 10–20 per cent. of the Mediterranean plants inoculated in his experiments gave abnormal reactions. It was explained to him in a letter from Prof. Mains that the variety hybridised freely and that the same trouble was experienced in America. In dealing with this variety, therefore, care was taken to use only seed from the original stock.

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[1] Since the present paper was written C. Sibilia has reported the determination of eight new physiologic forms in Italy, which have been designated Nos. 55–62 ("La specializzazione della *Puccinia triticina* in Italia", *Boll. Staz. Pat. veg. Roma*, xv (1935), 277).

Professor C. O. Johnston informs me in a letter, however, that he has already assigned Nos. 55–65 inclusive to physiologic forms determined by other workers.

Sources of cultures of Puccinia triticina

Of the collections of *P. triticina* which were sent to Cambridge or which I made myself, forty-six were tested. Most of these collections were obtained during the summers of 1932 and 1933.

Thanks are due to the mycologists who have sent collections of *P. triticina* from the following neighbourhoods: Aberystwyth (Mr D. Walters Davies); Newport, Salop (Mr N. G. Preston); Reading (Mr W. Buddin); Newcastle-on-Tyne (Dr F. T. Bennett); Cardiff (Mr J. Rees); Rothamsted (Miss M. D. Glynne); Newton Abbot (Mr A. Beaumont).

Collections of *P. triticina* from the above centres (except Rothamsted) were obtained on the following varieties of wheat which were grown from seed sent from Cambridge: Cambridge Rivet, Little Joss, Iron, Wilhelmina, April Bearded, and Red Marvel. Collections were also made on the University Farm at Cambridge from as many different wheat varieties as possible during 1932 and 1933. The majority of these were made in July and early in August, but additional collections were made when *P. triticina* appeared at other times of the year. For example, one new physiologic form (Form 68) was obtained once from a few uredospore pustules on volunteer wheat plants on the University Farm in February 1933.

I am also indebted to Dr d'Oliveira through whom three collections of *P. triticina* were obtained from near Lisbon, Portugal.

Forms 15 and 31 from Canadian collections were given to me by Dr Neatby of the Dominion Rust Research Laboratory, Canada, and were found useful for comparative purposes.

Stock cultures

The variety Wilhelmina was used for the maintenance of stock cultures throughout. Seedlings of this variety were inoculated with the rust samples as soon as they were received, and the culture was numbered and isolated under a lamp-glass.

Establishment of single-spore cultures

Of the forty-six cultures of *P. triticina* which have been tested in these experiments, thirty-one were derived from single spores. A few additional cultures were derived from single pustule isolations by the use of the method outlined by Johnston and Mains (8). It was not considered necessary to obtain single-spore cultures of the remaining mass cultures, as these gave the reactions typical of the physiologic form to which they have been assigned, without any evidence of mixture. Only a few of the collections as sent in from the field showed signs of being mixtures of physiologic forms. Single-spore isolations were invariably made from such collections.

Preservation of purity of cultures

All cultures of *P. triticina* were kept in the same greenhouse, each under a sterilised lamp-glass covered over the top with muslin. Great care was taken in renewing the stock cultures to prevent casual contamination. No mixture of individual cultures was observed when they were preserved in this way.

A disadvantage of keeping the stock cultures permanently under cover was the comparatively frequent necessity for renewal. A culture seldom lasted more than 3 weeks, and often, during hot weather, or during a period when *Erysiphe graminis* was prevalent, considerably less. The use of sulphur in the greenhouse was sometimes helpful in reducing the incidence of *E. graminis*.

In winter, during periods of fog and low light intensity, a few cultures (tested and untested) were lost through failure of infection.

Inoculation and incubation

The differential hosts were sown in pots with a sufficient number of seeds to produce eight to ten seedlings in each pot. The soil was a good loam obtained from the Cambridge Botanic Garden. Seedlings of the differential wheats were inoculated when in the two-leaf stage, the first leaf only being used. The eight pots of differential wheats were incubated together under a bell-jar on the floor of the greenhouse. When possible, two, three, or more sets of differentials were inoculated simultaneously with different cultures of *Puccinia triticina* for the purpose of comparison.

Time of experimentation and the number of times individual tests were made

With the exception of the months of December and January experiments were carried out with many of the cultures throughout the year from 1932 up to October 1934. Each culture was tested on the differential wheats five to ten or even more times. The best period for experiments with *P. triticina* was usually from May to September.

During the winter, especially in November and February, when the light intensity was low and dull weather prevalent, failure of infection sometimes resulted. Except under abnormally bad weather conditions, however, many of the physiologic forms produced as good reactions on the differentials in mid-winter as in the summer.

The incubation period of the cultures varied from 12 to 15 days in winter to 9 to 10 days in summer. Reactions were read as soon as the pustules were fully developed.

The effect of differences in light intensity and temperature on the reactions of individual physiologic forms will be dealt with later.

Effect of infection by Erysiphe graminis *on the reaction of resistant wheats to* Puccinia triticina

During these experiments it was frequently noticed that infection by *Erysiphe graminis* of a host usually very resistant to a certain form of *Puccinia triticina* induced the development of pustules of a very susceptible type. This was especially noticeable with Malakof wheat, and less frequently with Democrat and Webster. The occurrence of small localised groups of pustules on the leaves of these varieties was invariably associated with a mildewed area on the opposite side of the leaf, or actually over the pustules themselves; non-mildewed portions of such leaves exhibited the flecking characteristic of a type 0 reaction.[1]

INFECTION TYPES AND REACTION CLASSES

In the course of this study the five types of infection ranging from 0 (highly resistant) to 4 (very susceptible) were used, as previously described by Mains and Jackson (9).

Another type of infection, known as the "x" type, was described for *Puccinia graminis tritici* by Stakman and Levine (19) as: "Uredinia very variable, apparently including all types and degrees of infection on the same blade; no mechanical separation possible; on reinoculation small uredinia may produce large ones, and vice versa. Infection ill defined." This type of infection was occasionally encountered by Mains and Jackson (9), but was not used by them. It has since been used by Scheibe (17), Waterhouse (22), and Johnston and Mains (8). The "x" or "indeterminate" type of infection was encountered in this investigation. A variant of the type "x" reaction, which sometimes coincided with abnormally warm weather, was the occurrence of very numerous, small, crowded pustules on a leaf, the whole surface of which was extremely chlorotic.

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[1] Johnston (7) has drawn attention to a similar condition. He states that the normal reaction of Warden wheat to Form 9 of *P. triticina* is resistant, and the reaction commonly takes the form of a flecking of the leaves. When such leaves are infected with mildew, however, he has observed the development of pustules of a susceptible type in the chlorotic area immediately surrounding the mycelial weft, actually embedded in it, or in the chlorotic area on the surface of the leaf immediately opposite the mildew.

The "variable" or "intermediate" type of reaction (type 2–3), so described by Johnston and Mains (8) because it could not conveniently be placed either in a susceptible or a resistant category, was encountered.

The observed range in type of infection has been used throughout this investigation. This method of presenting data for the identification of physiologic forms agrees with that employed by Mains and Jackson (9), Scheibe (16, 17), and Johnston and Mains (8). Waterhouse (22) used mean infection types in his work on *P. triticina*, as did Stakman and Levine (20) for recording the reactions of the forms of *P. graminis tritici*.

The value of recording data by means of the observed range of infection types was mainly in recording the variable type 2–3 reaction, and in those cultures where variation between more than two degrees of infection occurred constantly and apparently regardless of seasonal changes. Examples of this type of infection are fairly numerous, *e.g.* the new Form 70 produces a type 0–2 reaction on both Hussar and Democrat. In this instance there is shown merely a fluctuation between the three resistant types of infection. Mains and Jackson (9) described a similar reaction for Form 11 on these two wheats. A more extreme variation in reaction is shown by the new Forms 69 and 70 on Malakof; these forms showed infection types of 2+, 3 and 4, which have consequently been described as 2–4. A similar reaction was described by Mains and Jackson (9) for Form 3 on Loros. Other examples of extreme variation in reaction, which, however, can be referred to environmental changes, will be discussed later.

EXPERIMENTAL RESULTS

Ten new forms of *Puccinia triticina*, eight of which were obtained in collections from England and Wales, and two from Portugal, are described in Table I. These new forms are numbered 66–75.[1] Included in the table is Form 15 (Scheibe (16)), which was isolated several times from Britain and once from Portugal, and a variation of Form 10 (Jackson and Mains (9)), which arose as a mutant of Form 66.

Relationships and variation of physiologic forms

In Table I it will be seen that the differences in infection type between some of the forms, *e.g.* 67, 71 and 73, are not clear-cut as between resistant and susceptible, but only between a variable or intermediate type 2–3 reaction, a susceptible reaction, and the indeterminate type "x" reaction.

[1] The international form numbers 66–75 were kindly assigned by Dr C. O. Johnston in January, 1935.

These differences in infection type, however, occur on more than one of the differential wheats, and slight though they appear to be they occur constantly.

Form 73 is clearly differentiated from Forms 67 and 71 by its production of a type x–4 reaction on Hussar. On this host the type "x" reaction occurred more frequently than the type 4 reaction. On the other hand, the reactions of Hussar to Forms 67 and 71 are clearly defined susceptible ones.

Table I

Reactions of the standard differential varieties to physiologic forms of Puccinia triticina *from Britain and Portugal*

Physiologic Form no.	Type of infection on wheat variety								Country of origin
	Malakof	Carina	Brevit	Webster	Loros	Mediterranean	Hussar	Democrat	
66	0	3–4	4	2–3	4	0–1	4	0–1	Britain
67	0	4	4	3–4	4	3–4	3–4	2–3 +	,,
68	0	3–4	4	3–4	4	0–1	– 1–2	0–1	,,
69	2–4	3	3–4	1–2	3–4	0–1	2–3	0–1	,,
70	2–4	2–4	3–4	3	3–4	0–1	0–2	0–2	,,
71	0	4	4	2–3	4	2–3	3–4	1–1 +	,,
72	0–1	3–4	4	2–3	4	0–1	x–4	0–1	,,
73	0	3–4	4	3–4	4	3–4	x–4 –	1–2	,,
74	0	0	0–1	0	2–3 +	0–1	0–1	0	Portugal
75	0	4	4	2–3	4	2–3	0–1 +	0–1	,,
15	0	0	0–1 +	0	0–1	4	0–1	4	Britain and Portugal
10 (British)	4	4	4	3	4	0–1	0–1	0–1 –	Britain

The variable type 2–3 reaction produced on Mediterranean by Form 71, as opposed to the susceptible type 3–4 reaction produced on that variety by Forms 57 and 64, is rather rare, as the only other form encountered in this investigation which produced such a reaction on Mediterranean is Form 75 from Portugual.

The variable type 2–3 reaction of Form 71 on Webster, again, is clearly opposed to the definitely susceptible reactions of Forms 67 and 73 on this variety. Finally, Form 67 is differentiated both from Forms 71 and 73 by the greater susceptibility shown to it by Democrat. The reaction (type 2–3 +) of Form 67 on Democrat is the most susceptible reaction produced on this variety by any of the new forms encountered in this investigation. This reaction was constant in the three cultures of *P. triticina* from different sources which were found to belong to Form 67, and a type 3–3 + reaction was encountered more often than a type 2 reaction.

Two of the physiologic forms isolated in England, viz. Forms 10 and 69, are very similar to certain previously described forms of *P. triticina*, though constant differences from such forms were observed in the reactions on several of the varieties. Table II shows a comparison between these two forms and some forms described by Mains and Jackson (9) and Johnston and Mains (8).

The English Form 10, which is a variation of Jackson and Mains' Form 10, constantly gave a lower degree of infection than the type form on four of the differentials. The English Form 10 is one whose origin will be discussed later; it originated in the greenhouse presumably as a mutant from a culture of Form 66.

Table II

Comparison between forms of Puccinia triticina *isolated in England and some forms previously described*

	Type of infection on wheat variety							
Physiologic Form no.	Malakof	Carina	Brevit	Webster	Loros	Mediterranean	Hussar	Democrat
10 (Mains and Jackson)	4	4	4	4	4	1–2	1–2	1–2
10 (English form)	4	4	4	3	4	0–1	0–1	0–1 –
43 (Johnston and Mains)	4	3	3	1–2	4	1	2	1–2
50 (Johnston and Mains)	4	2	4	2 –	3	0	2	0
69 (new form)	2–4	3	3–4	1–2	3–4	0–1	2–3	0–1

Form 69 is differentiated from Form 43 mainly by the extremely variable reaction on Malakof and by the variable (type 2–3) reaction on Hussar. It is distinguished from Form 50 in the same way, and also by the susceptible reaction on Carina.

It has already been recognised that certain physiologic forms are in reality closely related members of composite groups. Waterhouse (22) found that the addition of the wheat variety Thew to the differentials separated Form 26 into two forms. Scheibe (17) found that the addition of other varieties to the standard set of differentials would further subdivide Forms 11 and 13. Both these investigators were dealing with rust cultures which according to the usual differentials appeared to consist of a single form. A variant of this condition was described by Johnston and Mains (8). They found that several cultures, which according to their analytical key could be run down to certain physiologic forms, differed slightly but constantly from such forms. In their opinion these cultures indicated that such physiologic forms were in reality groups of forms. Several such cultures have been encountered in this investigation.

Form 15 was encountered in seven collections of *P. triticina* from Britain and in one from Portugal. In Table III it will be seen that certain single-spore cultures from these collections, viz. nos. 34 Portugal, 57 Cambridge, 54 Cambridge, and 37 Cardiff, agree very closely with Form 15 described by Scheibe (16). On the other hand, in four other cultures, viz. nos. 38 Cardiff, 36 Cardiff, 42 Newport, and 44 Devon, slight divergences from Form 15 were recorded in the reactions on Brevit and Hussar. Cultures no. 38 Cardiff and no. 42 Newport are alike in the greater susceptibility shown by Brevit, and cultures no. 44 Devon and no. 36 Cardiff resemble one another in the greater susceptibility shown to them by Hussar. This seems to indicate that Form 15 exists in Britain in association with at least two closely allied forms. For purposes of classification in this paper, however, these cultures have been referred to Form 15.

Table III

Comparison between physiologic Form 15 *and certain cultures identical with or closely related to it*

Culture no.	Physiologic Form no.	Type of infection on wheat variety							
		Malakof	Carina	Brevit	Webster	Loros	Mediterranean	Hussar	Democrat
—	15	0	0	0–1	0	0–1	4	0–1	4
No. 34 Portugal	—	0	0	0–1	0	0–1	4	0–1	4
No. 57 Cambridge	—	0	0	0–4	0	0–1	4	0–1	4
No. 54 ,,	—	0	0	1	0	1	4	1 –	4
No. 37 Cardiff	—	0	0	0–1 +	0	0–1	4	0–1	4
No. 38 ,,	—	0	0	2–2 +	0	0–1 +	4	1–1 –	4
No. 42 Newport	—	0	0	0–2 +	0–1 –	0–1 +	4	0–1 +	4
No. 44 Devon	—	0	0	1–1 +	0	1 –	4	0–2	4
No. 36 Cardiff	—	0	0	0–1 –	0	1–1 –	4	1–2 –	3–4

Group susceptibility and resistance of differential wheats

It can be seen from Table I that the varieties Democrat and Malakof show a very high degree of resistance to the great majority of the cultures that have been tested. With the exception of Form 15 Democrat was resistant to ten of the forms, that is, to all except Form 67, to which it exhibits the intermediate type 2–3 reaction.

Malakof was resistant to nine of the twelve forms, and except for the type 0–1 reaction shown by Form 72, the degree of resistance of this variety was invariably very great. It frequently happened in an otherwise normal experiment that some of the inoculated Malakof seedlings appeared not to have "taken", *i.e.* they exhibited an immune reaction. This tendency was particularly noticeable in experiments made during the winter. Apart from this, the normal resistant reaction of Malakof usually took the form of a very faint flecking of the leaves. For this reason, when this variety was infected by mildew, the production of groups of type 4 pustules was the more striking.

Mediterranean was resistant to seven of the forms, but proved a valuable variety for differentiation owing to its clearly defined reactions.

The variety Hussar showed marked variability in its reactions to the forms with which it was inoculated, and was, to a great extent, the only variety whose reactions were seriously affected by seasonal changes. Mains and Jackson (9) and Waterhouse (22) have recorded that this wheat is very sensitive to seasonal changes, often being highly resistant to a certain form of *P. triticina* during the autumn and winter, and only moderately or slightly resistant in late spring. In this investigation, variation in reaction in Hussar could often be traced to seasonal changes, but some variations of a profound character could not be explained in this way.

Webster was remarkable for the frequency with which it exhibited the variable type 2–3 reaction, and was highly resistant to one new physiologic form only, viz. Form 74.

Carina, Brevit, and Loros wheats exhibited a uniformly high degree of susceptibility to all but one of the new forms. The reactions on these three varieties were, in fact, identical except in one particular, which was in the type 2–3 reaction of Form 74 on Loros, whereas the reactions on Brevit and Carina were type 0 and type 0–1 respectively. To the remaining forms these three varieties were susceptible to the same degree.

It is interesting to compare the high percentage of resistant reactions (types 0–2) of the Portuguese Form 74 on the differentials, with the correspondingly rather high percentage of susceptible reactions (types 3–4) exhibited by most of the British forms.

ORIGIN OF A MUTANT FROM CULTURE NO. 59 NORTHUMBERLAND

In August 1933 samples of *P. triticina* were received from the vicinity of Newcastle-on-Tyne. Stock cultures were established on Wilhelmina wheat, and the cultures were numbered 58 Northumberland and 59 Northumberland. The wheats from which they were obtained were Red Marvel and Little Joss respectively.

In these experiments it is emphasised that inoculum for the differentials was invariably taken from the stock cultures which were covered by lamp-glasses with spore-proof tops, and that mixture of the cultures was never observed.

In Table IV details of all the tests which were made with culture no. 59 Northumberland are presented. The temperatures given are the average maximum daily temperatures during the incubation period of the culture. To obtain an average of the hours of sunshine per day the total number of hours of sunshine during the whole incubation period was divided by the number of days. There may have been one or two consecutive days during the incubation period entirely without sunshine. This frequently happened in winter and early spring.

In Table IV it is seen that experiments with culture no. 59 North. were commenced a month after it was received from the field. In the first four tests on the differentials this culture was used as it was obtained from the field, *i.e.* it was not a single-spore culture. The culture, however, appeared to be pure, and it is believed that it was so.

In the first test the reaction of Malakof to culture no. 59 North. was type 0 on eleven plants, and of Hussar was type 4 on eight plants. In the four succeeding tests the reaction of Hussar to this culture became type 0–1, afterwards reverting to the original type 4 reaction. The aberrant behaviour of the reaction of Hussar to this culture was noticed in several other cultures of the same physiologic form (Form 66) and will be discussed in a later section.

A single-spore culture was obtained from the mass stock culture no. 59 North. in November 1933. In a test with this single-spore culture in March 1934, during a period of abnormally low temperature and low light intensity, a type 4 reaction was produced on Malakof. Spores obtained from this abnormal type 4 reaction were reinoculated at once on fresh Malakof seedlings, and a single pustule was selected from the resulting type 4 infection and formed the basis of the mutant culture no. 60 North. Experiments with culture no. 60 North. were then carried on concurrently with single spore culture no. 59 North. Table IV shows that culture no. 60 North. continued to produce a type 4 reaction on Malakof and on Hussar a type 0–2 reaction instead of the type 4 reaction of the parent culture no. 59 North. For comparison the two cultures no. 59 North. and no. 60 North. were tested simultaneously on sets of differentials in May, June, and October. The reactions can be compared in Table IV.

A description of the mutant culture no. 60 North. was sent to Dr C. O. Johnston, who regards it as a variation of Form 10 (Mains and Jackson (9)). The mutant culture differs from Form 10 only in a slightly lower degree of susceptibility on four of the differentials (see Table II).

It may be noted in Table IV that in the test of culture no. 60 North.

Table IV

Reactions of differential varieties to culture no. 59 Northumberland and a mutant culture obtained from it

Culture no.	Date	Average no. of hours of sunshine per day during incubation	Av. max. daily temperature during incubation (° F.)	Type of infection on wheat variety								
				Malakof	Carina	Brevit	Webster	Loros	Mediterranean	Hussar	Democrat	
59 North. (mass culture) (single-spore culture)	5. ix. 33	8 +	82	0 (11)	4	4	2	4	1 +	4 (8)	0	Form 66
	18. ix. 33	6 –	75	0 (6)	4	4	3	4	0–1	1 (5)	0–1 –	
	18. ix. 33	6 –	75	0 (7)	4	4	3–3 +	4	1	1 (4)	0–1 –	
	30. ix. 33	3 +	70	0 (5)	3	4	2 +	4	0–1	0–1 (7)	0–1 –	
	1. iii. 34	3 –	68	*4 (3)	3	4	3 –	4	0	1 (5)	1	
	19. iii. 34	3 –	70	0 (6)	2 +	4	2 +	4	0	4 (5)	0	
	19. v. 34	8 +	73	0 (8)	3	4	2 +	4	1	4 (7)	0	Form 66
	19. vi. 34	8	79	0 (7)	–	–	–	–	–	4 (7)	–	
	6. vii. 34	9 +	79	0 (7)	4	4	3	4	0–1	4 (16)	1 –	
	3. x. 34	?	68	0 (7)	4 –	4	3 +	4	0–1	4 (8)	1	
60 North. (single-pustule culture from*)	5. iv. 34	4 –	70	4 (9)	4	4	3	4	0	0 (7)	0	
	19. v. 34	8 +	73	4 (6)	4	4	3	4	1	1 (4)	–	
	19. vi. 34	8	79	4 (9)	–	–	–	–	–	1 + (6)	–	Form 10
	25. vi. 34	5 +	79	4 (7)	4	4	3	4	0–1	1 (8)	1 –	
	10. vii. 34	13	85	4 (5)	4	4	3	4	0–1	2 (6)	1 –	
	3. x. 34	?	68	*x* (4) 4 (8)	4	4	3 +	4	0	0 (6)	1 +	

* Rust from these Malakof plants was grown on other Malakof plants. A single pustule was selected from the resulting infection and formed the basis of the culture No. 60 Northumberland.

Numbers in brackets indicate the number of plants infected.

The single-spore culture from No. 59 Northumberland was established in November 1933.

during July 1934 some of the Malakof seedlings exhibited a type *x* reaction. This reaction and the slightly more susceptible reaction than usual of Hussar coincides with an abnormally high average maximum temperature during the incubation period.

The origin of Form 10 (culture no. 60 North.) in Cambridge by mutation from Form 66 seems to be clear. The only other explanation of its appearance, viz. that it might have arisen as a casual contamination, may be discarded for the following reasons:

(1) The mutation was first observed in March. At that time of the year *P. triticina* is very rare in the field if it exists at all. Since 1931 it has not been observed in that month. In addition, this variation of Form 10 was not isolated in any field collection in Britain.

(2) Only two physiologic forms (Forms 69 and 70) in culture in the greenhouse at the time when the mutant Form 10 arose produce a susceptible type 4 reaction on Malakof (see Table I). The reactions of both these forms on Malakof, however, are extremely variable. The mutant Form 10, on the other hand, has consistently produced a type 4 reaction throughout a somewhat considerable range of temperature and light intensity (see Table IV). In addition, Form 70, which it more closely resembles, was tested simultaneously with the mutant form in July 1934, when the average maximum daily temperature was abnormally high. As a result of this high temperature an abnormal type 4 reaction was produced on Hussar by Form 70, and the resistant reaction, was, as usual, produced by the mutant Form 10. Thus these two forms also differ in their reaction to environment, and the possibility that spores from Form 70 had become mixed with the single spore culture no. 59 North. (Form 66) can be ruled out.

Mutations in rust fungi have occasionally been reported. Stakman, Levine and Cotter (20) report that in a culture of Form I of *P. graminis tritici*, which had been constant pathogenically in the greenhouse for 13 years, there suddenly appeared a new form (afterwards known as Form 60), which was different pathogenically from anything they had hitherto known. The constancy of this new form was proved by extensive inoculations. A feature of this mutant was its inability to produce normal infection on two of the differentials. Two other mutants that arose from Form I, viz. Forms 21 and 17, proved to be much more virulent than the original culture. Later, still another form arose from the original culture, which, though not fully identified by the writers, seemed to be different from anything hitherto described. This additional mutant was designated as Form 58.

Though evidence of mutation in pathogenicity in *P. graminis tritici* is rare several examples of mutation in spore colour of this rust have been described by Newton and Johnston (13) and by Waterhouse (22).

The only reference to mutation in *P. triticina* hitherto presented is that by Johnston (6). He described an aberrant form of *P. triticina* (Form 27) which was assumed to be of mutational origin. This form, which was collected in nature, differed from all known forms in the length of the incubation period, the small size of the uredospore pustules, and the light orange colour of the spores.

In view of the mutational changes in rust fungi just described, the origin of culture no. 60 North. (Form 10) by mutation from culture no. 59 North. (Form 66) seems probable.

APPARENT INSTABILITY IN REACTION OF CERTAIN CULTURES OF FORM 66 ON HUSSAR

In Table IV it is seen that in the first test on the differentials with culture no. 59 North. Hussar exhibited a type 4 reaction. In the next four tests, three of which were made in the same month as the first, the reaction changed to type 0–1. In the test succeeding the one in March 1934 that revealed the mutant Form 10 (culture no. 60 North.) the reaction again became type 4 and remained constant to this type during subsequent tests. Culture no. 58 North., which was sent to me at the same time as the above, gave a similar type 4 reaction on Hussar. On the strength of this susceptible reaction on Hussar both these cultures have been identified as belonging to Form 66.

Similar behaviour of other cultures of Form 66 has frequently been observed. The reactions of three such cultures are illustrated in Table V.

In each example in this table there is shown an abrupt change in reaction on Hussar from resistant to susceptible, exactly similar to that observed in culture no. 59 North., as shown in Table IV. In these cultures, however, the reverse change in reaction from susceptibility to resistance, also evident with culture no. 59 North., has not been observed. It is thought that the change may be a reversible one, and not simply a change in reaction from resistant to susceptible as exhibited by the three cultures referred to in Table V.

That environment is not the cause of the change in reaction on Hussar of culture no. 59 North. (Table IV) or in the cultures whose reactions are illustrated in Table V, can be seen in Tables IV and V. Slight fluctuations in reaction can usually be explained by some environmental factor. For example, the type 2+ reaction of culture no. 8 Cardiff in June 1933 coincided with an abnormally high average maximum temperature during incubation. This is a common effect of high temperature on some physiologic forms. The major fluctuations in reaction, however, cannot be explained as being due to environment. For example, culture no. 51 Reading, at an average maximum temperature of 79° F., produced a type 1 + reaction on Hussar in August 1933. In the same month of 1934 at virtually the same temperature it produced a type 4 reaction. Further, culture no. 23 Newport, under the worst conditions for experimenting with physiologic forms, *i.e.* very low light intensity and unusually low temperature, produced a type 4 reaction in February 1934. This same reaction was produced by this culture in a heat wave during the previous August (1933), and under the typically optimum conditions of the following June (1934).

It is not believed, therefore, that environmental fluctuations can account for the aberrant reactions of Hussar to the cultures belonging to Form 66 indicated in this discussion.

The behaviour of the cultures of this form cannot be clearly explained at present. It seems to point, however, to some inherent instability in Form 66. As this phenomenon has been observed in four[1] cultures from widely different sources it is believed to be somewhat typical of the form. Such divergences of reaction, other than those which can readily be explained by environmental fluctuations of light and temperature, have not been observed in any other physiologic form.

Belief in the stability and fixity of individual physiologic forms is widely held. Stakman (18) summed up the general view on the matter as follows: "One of the most remarkable things about the varieties and physiologic forms of *P. graminis* is the absolute certainty with which they can be determined by their pathogenicity on selected host plants."

In this investigation, however, while the results in general indicate that the great majority of physiologic forms are stable in their reactions, experiments with certain cultures of Form 66 show that instability in reaction, apparently inherent, does exist. Though such instability is probably of rare occurrence, the possibility that it may be encountered in tests with physiologic forms should not be overlooked.

The study of the cultures belonging to Form 66 indicates the desirability of testing physiologic forms repeatedly on the differential varieties. Attention is drawn to the fact that by the resistant reaction on Hussar,

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[1] The same phenomenon has been observed in two other cultures which are thought to belong to Form 66, but which were lost before a sufficient number of tests were made with them on the differentials.

Table V

Showing the reactions of three cultures of Puccinia triticina *on Hussar*

Date	Temperature	Culture no. 23 Newport	Temperature	Culture no. 8 Cardiff	Temperature	Culture no. 51 Reading
August 1932	—	—	76	1 (mass culture)	—	—
October 1932	70	1–2 (mass culture)	—	—	—	—
March 1933	72	1 (single spore culture)	72	1 ,,	—	—
April 1933	74	2 ,,	—	—	—	—
May 1933	76	2 ,,	76	1 ,,	—	—
June 1933	—	—	80	2 + ,,	—	—
August 1933	84	4 (single spore culture)	78	4 ,,	79	1 + (mass culture)
September 1933	—		75	4 ,,		
February 1934	69	4 ,,	68	4 (single spore culture)		
March 1934	67	4 ,, } Form 66		} Form 66		
March 1934	72	4 ,,			77	3 + ,,
June 1934	77	4 ,,			79	4 ,,
July 1934	83	4 ,,	77	4 − ,,	78	4 (single spore culture) } Form 66
August 1934			high	4 ,,		
August 1934					78	4 ,,

Temperature = Av. max. daily temperature (° F.) during incubation period.

The change in reaction of these cultures was a clear-cut one, unaccompanied by any sign of mixture of two forms.

culture no. 59 North. (Table IV) and the cultures indicated in Table V would have constituted another new physiologic form if the reaction on Hussar had remained constant to that type of infection. It is not unlikely that such a new form would erroneously have been described if only a few tests with these cultures had been made on the differentials and the cultures lost or discarded before their aberrant behaviour on Hussar became apparent.

EFFECT OF ENVIRONMENTAL FACTORS ON THE BEHAVIOUR OF PHYSIOLOGIC FORMS OF *PUCCINIA TRITICINA*

It has been mentioned earlier in this paper that experiments with many of the cultures of *P. triticina* were carried on from February 1932 until September 1934, throughout the year with the exception of the months of December and January.

Mains and Jackson (9) and Johnston and Mains (8) agree in having carried out tests on physiologic forms of *P. triticina* only in winter and early spring. Waterhouse (22) experimented on two physiologic forms during summer and winter in order to test their variation in behaviour at different times of the year. Scheibe (16) conducted tests in summer and winter, and though he does not recommend experimenting in midwinter he states that he obtained good results then during bright frosty weather.

Johnston and Mains (8) state: "It is now a well-known fact that environmental conditions often have a marked effect on the type of reaction exhibited by a variety."

Mains and Jackson (9) found that environment influenced the reaction of Hussar to some physiologic forms to the extent that this variety was highly resistant when inoculated in autumn and winter, and only moderately or slightly resistant when inoculated in late spring. Waterhouse (22) found that certain varieties of wheat were susceptible to two physiologic forms of *P. triticina* in summer and resistant in winter.

Johnson (5) made a study of certain forms of *P. graminis tritici* which produce the type "*x*" reaction on certain wheats at ordinary greenhouse temperatures (about 65° F.). He found that Mindum seedlings inoculated with Form 29 at 58° F. gave a highly resistant reaction, and at 73° F. a susceptible reaction. He states that this behaviour is typical of those forms which at 65° F. produce an "*x*" reaction. Increased light intensity was found by Johnson to produce the same effect as increased temperature on the development of those forms which produce the "x" reaction. He concludes that it is of the greatest importance to avoid excessively high or excessively low temperatures in greenhouses where physiologic forms are being identified.

Gassner and Straib (3) showed in investigations at Brunswick on Form 14 of *P. triticina* that at very low temperatures some of the reputedly highly resistant varieties, such as Malakof, Norka, Democrat and Mediterranean, became very susceptible. Webster, on the other hand, maintained its resistance at low temperatures.

The question arises, therefore, whether physiologic form surveys conducted in different seasons and possibly under different conditions by various workers, can be correlated satisfactorily one with another.

In the present study of physiologic forms of *P. triticina*, the results indicate that while some forms vary somewhat in their reactions, especially on the variety Hussar, many of the forms do not differ appreciably in winter and summer tests.

In this study of physiologic forms it is to be noted that critical experiments to test the effect of environment on host reactions to physiologic forms of *P. triticina* have not been carried out. Daily records of greenhouse temperatures were kept throughout the year, however, and it was usually found that an atypical reaction on a certain host by a physiologic form could be correlated with some definite fluctuation from the normal average temperature during the incubation of that form.

The effect of low light intensity on the reactions to physiologic forms was found to be generally the same as that of low temperature. A gradual increase in susceptibility as the season advanced, however, was sometimes undoubtedly due more to increase in light than to increase in temperature. The relative importance of these two factors, however, in their effect on host susceptibility was generally difficult to estimate.

The greenhouse in which these experiments were conducted was heated during the winter, but thermostatic temperature control was not available. The average minimum temperature ranged from about 55 to 60° F. Winter experiments in which complete failure of infection resulted after inoculation are thus thought to be less the effect of low temperature than of extremely low light intensity. Such experiments usually coincided with prolonged dull periods. That the temperatures mentioned above (55–60° F.) were not in themselves too low for development of *P. triticina* given a sufficiently high light intensity is shown by the experiments of Gassner and Straib (3), who obtained a successful infection with Form 14 at temperatures of from 8 to 12° C. (46·4–53·6° F.).

In summer the glass of the greenhouse was whitewashed so that the range of temperature was reduced to some extent. Maximum daily temperatures were often considerably higher than those prevailing during the greater part of the day, and usually persisted only for a short time.

In Tables VI, VII and VIII averages of the number of hours of sunshine per day experienced during the incubation periods are usually given. These records are not felt to be of any great value as accurate indications of light intensity during an experiment, as the periods of sunshine in winter were so unequal in their distribution. They serve to indicate to some extent, however, the very small amount of actual sunshine sometimes experienced during the winter. Total light radiation during the tests rather than the duration of bright sunlight would probably be a more accurate guide to the effect of light on the behaviour of the physiologic forms.

Abnormal production of the type "x" reaction

An abnormal type "*x*" reaction was produced by some physiologic forms at unusually high temperatures. An example of this is shown in Table VI by Form 70 on Malakof, on which the normal reaction of this form was a variable type 2–4. On the occasion when the "*x*" reaction was produced the average maximum temperature during the incubation period was 85° F., and the light intensity was correspondingly high.

Abnormal type "*x*" reactions were also produced by two physiologic forms as a result of decrease in temperature combined with low light intensity.

It will be seen from Table VI that the normal reaction of Form 73 on Webster was type 3–4. The records of the reactions of this variety to Form 73 are thought to indicate the effect on rust development of gradually decreasing light intensity in late autumn followed by an increase in intensity in early spring. The tests recorded at the beginning of October 1933, in the middle of that month, and at the end of it, show the decreasing series of reactions indicated by the reaction types 4, 3+ and 2+. The next test made in February 1934 produced the type "*x*" reaction. The type 4 reaction was again produced in March and in subsequent tests in June, July and August. In this instance it is difficult to determine whether low temperature or low light intensity was the main contributory cause of the decrease in susceptibility, with the final production of the type "x" reaction. It does not appear from the temperatures shown in Table VI that temperature can account for the change in reaction. The decreasing series of types of reaction of Form 73 on Webster, however, are thought clearly to suggest that decrease in susceptibility followed decrease in radiation as winter conditions set in.

Form 71, the reactions of which on Hussar are shown in Table VI, produced a type "x" reaction in a test in March 1934. The normal reaction, as indicated by tests in August 1933, and June and August 1934, was type 3–4. The production of the type "x" reaction in this instance seems to be clearly associated with the lower temperature and lower light intensity of March as compared with those of the other months.

Table VI

Abnormal production of type "x" reaction by certain forms under certain environmental conditions

	Date	S.	T.	Reaction
Form 70 on Malakof:	April 1933	7+	76	4−
	May 1933	5−	76	2+
	June 1933	9−	76	2+
	August 1933	7−	83	3−
	September 1933	7+	79	4
	February 1934	4	69	4
	July 1934	13	85	*x
	Normal reaction type 2–4			
Form 71 on Hussar:	August 8, 1933	8−	81	4−
	August 11, 1933	6+	82	4
	August 21, 1933	8+	73	4
	March 1934	3	70	*x
	June 18, 1934	8+	77	3+
	June 27, 1934	5+	78	3
	August 1934	High	High	3
	Normal reaction type 3–4			
Form 73 on Webster:	October 4, 1933	3−	68	4
	October 16, 1933	4	72	3+
	October 31, 1933	2+	69	*2+
	February 1934	3	69	*x
	March 1934	3	68	4
	June 1934	8	79	4−
	July 1934	High	78	4−
	Normal reaction type 3–4			

Date = Date on which reactions were read.
S. = Av. number of hours of sunshine per day during incubation.
T. = Av. max. Fahrenheit temperature during incubation.
* = Abnormal reaction.

Increase in resistance of normally susceptible varieties associated with low light intensity and low temperature

Increase in resistance in normally susceptible reactions of some wheat varieties to certain physiologic forms was frequently noticed in experiments during the winter. Three examples of such increase in resistance are shown in Table VII.

With Form 72 the normal reaction on Hussar varied from the indeterminate type "x" to susceptible. A very resistant type 1 reaction was produced on Hussar by this form in a test in November 1932, when the average temperature was lower than usual and the light intensity extremely low, there being an average of little more than 1 hour of sunshine per day during the incubation period.

The susceptible type 4 reaction of Form 73 on Carina was replaced by a type 2 reaction in a test at the end of October 1933. In the greater light intensity of February 1934, but at the same temperature, 69° F., the reaction of Carina to this form became type 3 +. The type 4 reaction was again obtained in the following month at an average temperature of only 68° F. The increase in resistance of Carina to Form 73 thus seems to be connected more with low light intensity than with low temperature.

Table VII

Influence of environmental factors on increase in resistance of normally susceptible varieties

	Date	S.	T.	Reaction
Form 72 on Hussar:	November 1932	1 +	68	*1
	June 10, 1933	11 +	74	4
	June 24, 1933	6 +	76	*x*
	July 1933	8 −	72	*x*
	August 1933	7	83	4
	March 1934	3 −	68	*x*
	July 1934	High	78	*x*
	Normal reaction type *x*–4			
Form 73 on Carina:	October 4, 1933	3 −	68	4 −
	October 16, 1933	4	72	4 −
	October 31, 1933	2 +	69	*2
	February 1934	3	69	3 +
	March 1934	3 −	68	4
	July 1934	High	78	4
	August 1934	High	79	4 −
	Normal reaction type 4			
Form 75 on Carina:	September 1933	9	82	4
	February 1934	3	69	*2 +
	March 1934	3	68	4 −
	March 1934	3 +	70	4
	July 1934	High	78	4
	Normal reaction type 4			

S. = Av. hours of sunlight per day during incubation.
T. = Av. max. daily temperature during incubation.
* = Abnormal reaction.

The reaction of Carina to Form 75 under low light intensity, also shown in Table VII, is similar to that of Form 73. The normal type 4 reaction was changed in February 1934 to a type 2 reaction. A test in the following month again yielded the type 4 reaction. In this example, also, low light intensity rather than low temperature seemed to be the cause of the increase in resistance.

Examples of increase in resistance in winter in the reactions of varieties which were normally susceptible to cultures of *P. triticina* are comparatively numerous, and are all more or less similar to those illustrated in Table VII. It is thought, however, that while in some of the examples there is a definite correlation between increase in resistance with relatively low temperatures, in general the winter temperatures in the greenhouse were not low enough to inhibit normal development of cultures if the light intensity had been normal. For example, a prolonged dull period in March 1934 at an average maximum temperature of 70° F. resulted in the production of a type 2+ reaction by culture no. 59 North. on Carina, the normal reaction being type 3–4. Earlier in the same month, but in better light, the reaction of Carina to this culture had been type 3 (Table IV).

Increase in susceptibility of normally resistant varieties associated with increased temperature and light intensity

Increase in susceptibility in some varieties normally resistant to certain physiologic forms was noticed in association with increased temperature and light intensity. Such increase in susceptibility, however, was not usually of such a character as to cause a complete change in reaction from resistant to susceptible. A striking exception to this was observed in the reactions of Form 70 on Hussar (see Table VIII). The normal reaction of Hussar to this form was type 1–2. In two tests, however, in August 1933 and July 1934, when the average maximum temperatures during incubation were abnormally high (83 and 85° F. respectively), type 4 reactions were produced on this variety. The correlation shown in this example between high temperature and increase in susceptibility is very clear. A somewhat similar increase in susceptibility at a very high temperature was shown by a culture (no. 31 Cambridge) of Form 68 on Hussar.

The reaction of Form 68 (culture no. 35 Cardiff) on Hussar, which was normally type 1–2, shows a variation apparently due to change in light intensity somewhat similar to the examples given in Table VII. With this culture Hussar becomes more susceptible with increasing light intensity, a type 0 reaction having been produced in March 1934, and a type 1–2 reaction in April, June and July.

On the whole, forms which produced a resistant reaction on the differential varieties were observed to vary less with changes in environment than those which produced susceptible reactions. In the cultures whose reactions on Hussar are given in Table VIII, the variation of the two cultures of Form 68 is not very great, and the profound variation in the reaction of Form 70 on Hussar was an isolated example. In general, physiologic forms which produced resistant reactions on the differential varieties were very constant to their particular types of infection in tests made at different times of the year.

Table VIII

Effect of increase in light intensity and temperature on the reactions of varieties normally resistant to certain forms of Puccinia triticina

	Date	S.	T.	Reaction
Form 70 on Hussar:	April 1933	7+	76	2−
	May 1933	4+	76	2
	July 1933	7	72	2
	August 1933	6+	83	*4
	September 1933	7	75	1+
	February 1934	4	69	1−
	July 1934	13	85	*4
	Normal reaction type 1–2			
Form 68 on Hussar (culture no. 35 Cardiff):	March 1934	3+	72	0
	April 1934	4	71	2
	June 2, 1934	7	74	1
	June 15, 1934	8	77	2
	July 1934	High	78	2
	Normal reaction type 1–2			
Form 68 on Hussar (culture no. 31 Cambridge):	April 1934	4	70	1
	June 1934	8	79	1+
	July 3, 1934	7	77	1
	July 16, 1934	High	83	*2
	August 1934	High	76	1
	September 1934	Low	Low	0
	Normal reaction type 0–1			

S. = Av. number of hours of sunshine per day during incubation.
T. = Av. max. daily temperature during incubation.
* = Abnormal reaction.

General trend of variation in reaction

Attention is drawn to the fact that all the examples of variation in reaction cited in the foregoing discussion which have been considered to be due to environmental factors have been in the direction of decrease in susceptibility with decrease in temperature and light intensity. The previously mentioned observations of Jackson and Mains (9), Waterhouse (22), and Johnson (5), support this view.

Petursen (14), in experiments on *P. coronata* var. *avenae*, inoculated physiologic Forms 1, 3, 4 and 7, on the differential host varieties at temperatures 57, 70 and 77° F. He found that the type of reaction produced by Forms 1 and 2 was not influenced by temperature. Red

Rustproof was susceptible to Form 4 at 70 and 77° F. but resistant to it at 57° F. Form 7 developed heavily on all the differential varieties at 77° F., but at 57° F. it failed to produce spores on Green Mountain, White Tartar, and Green Russian Oats.

Gordon (4), in experiments on *P. graminis* var. *avenae*, found that Joanette oats was highly resistant to Forms 1, 3, 4 and 5 at 57·4° F., but at 71·9 and 64·8° F. this variety was completely susceptible to all these forms.

Change of reaction from resistant to susceptible at low temperatures such as that described by Gassner and Straib (3) for Form 14 of *P. triticina* has not been observed in the present investigation. These workers found that certain wheat varieties, namely Norka, Malakof, Democrat and Mediterranean, which were very resistant to Form 14 at 18·7° C., became very susceptible at temperatures of 8–12° C.

When a change in reaction from susceptible to resistant was observed in my experiments, however, the decrease in susceptibility at the lower temperatures was invariably associated with low light intensity, and the results indicate that in many instances low light intensity was the more important contributory factor to the change in reaction. Temperatures as low as those at which Gassner and Straib worked (8–12° C.) were never experienced in the greenhouse. The observations which I have made, however, indicate that, in general, low temperature and low light intensity reduce the susceptibility of certain of the differential varieties to many physiologic forms of *P. triticina*.

DISTRIBUTION AND PREVALENCE OF PHYSIOLOGIC FORMS OF *PUCCINIA TRITICINA* IN ENGLAND AND WALES

The localities from which the cultures tested in this study were collected are fairly representative of England and Wales, although the north of England is represented only by collections obtained from the vicinity of Newcastle-on-Tyne. Also, from some of these localities only one collection was obtained, *e.g.* Rothamsted and Aberystwyth, so little is known of their representative rust populations.

Table IX shows the geographical distribution in this country of the physiologic forms which have been determined, and to some extent also their annual distribution and relative prevalence.

Only a few of the forms described for England and Wales were common to two or more localities. Of the ten physiologic forms shown in the table, five were obtained once only. These were Forms 10, 69, 71, 72 and 73. Form 70 was isolated twice, but from the same locality in successive years. Form 68 was isolated three times, Form 67 five times, Form 15 seven times, and Form 66 fourteen times.

Table IX

Physiologic forms obtained in England and Wales during 1931, 1932 *and* 1933

Rothamsted	1931	—	66 (1)	—	—	—	—	—	—	—	—
Cambridge	,,	—	66 (1)	—	—	—	—	—	—	—	—
Cambridge	1932	—	66 (4)	—	—	—	—	—	—	—	—
Newport (Salop)	,,	—	66 (1)	67 (1)	—	—	—	—	—	—	—
Newton Abbot (Devon)	,,	—	—	—	68 (1)	—	69 (1)	—	—	72 (1)	—
Cardiff	,,	—	66 (1)	—	—	—	—	70 (1)	—	—	—
Reading	1933	—	66 (1)	67 (1)	—	—	—	—	—	—	—
Newcastle-on-Tyne	,,	—	66 (2)	—	—	*10 (1)	—	—	—	—	—
Ashmore (Dorset)	,,	—	—	67 (1)	—	—	—	—	—	—	—
Aberystwyth	,,	—	—	—	—	—	—	—	71 (1)	—	—
Cambridge	,,	15 (2)	66 (3)	67 (1)	68 (1)	—	—	—	—	—	—
Newport (Salop)	,,	15 (1)	—	—	—	—	—	—	—	—	—
Newton Abbot (Devon)	,,	15 (1)	—	—	—	—	—	—	—	—	73 (1)
Cardiff	,,	15 (3)	—	67 (1)	68 (1)	—	—	70 (1)	—	—	—
Total no. of times each form was isolated	1931	—	2	—	—	—	—	—	—	—	—
	1932	—	6	1	1	—	1	1	1	1	—
	1933	7	6	4	2	1	—	1	—	—	1

* Mutant from culture no. 59 North. (Form 66).

( ) Numbers in brackets indicate the number of collections from which the form was isolated.

FIELD OBSERVATIONS ON THE OCCURRENCE OF *PUCCINIA TRITICINA* IN THE VICINITY OF CAMBRIDGE

Evidence of overwintering of *P. triticina* in Britain has been presented by Mehta (10). In the course of this survey *P. triticina* was found in the field during 1932 from early July till October. It was not again found until February 1933, when a few pustules were seen on self-sown wheat.

From observations made during 1932, 1933 and 1934 it appeared that *P. triticina* did not become generally prevalent in the field until June or July. In 1933 it was fairly common late in June, and by the middle of July it had established itself on most of the varieties kept under observation. In 1934 the experimental plots still showed only slight traces of *P. triticina* by the middle of July, shortly after which the plots were harvested.

In general, field observations at Cambridge indicate that by the time *P. triticina* becomes prevalent in most seasons many wheat varieties have already been infected so heavily by *P. glumarum* that most of the leaves have already shrivelled. (This generalisation does not hold good for 1934, which was an abnormal season for cereal rusts. Most wheat varieties on the experimental plots were only slightly infected with

P. glumarum.) In a fine summer harvest follows soon after general infection by *P. triticina*. Teleutospores were not observed by the writer in the field.

GENERAL DISCUSSION

It is recognised that the data which have been presented on the distribution of physiologic forms of *P. triticina* in Britain are incomplete. The results obtained, however, are sufficient to indicate that British forms differ markedly from those on the continent of Europe and in North America. Form 66, which is comparatively virulent on the differential varieties, appears to be the most widely distributed as well as the most frequently encountered form of *P. triticina* present in Britain.

The results of this survey in general indicate that the number of hitherto undescribed forms of *P. triticina* which have been encountered is very high for an area as small as England and Wales. It might have been expected that British physiologic forms would be mainly identical with those previously described in continental Europe. On the contrary, Form 15 is the only one isolated in Britain which agrees with any form hitherto described there. This form has a very wide distribution. It was first described by Scheibe (16) from Germany. Wellensiek (23) encountered it in the Netherlands, and Dodoff (2) in Bulgaria; Radulescu (15) isolated it from a collection of *P. triticina* obtained from Greece, and Johnston and Mains (8) record it in the United States. Finally, in the present survey this form has been shown to occur also in Portugal.

The variant of Form 10, which arose as a mutant from a culture of Form 66, agrees essentially with Form 10 described from the United States.

With regard to the fact that no other form except Form 15 has been encountered in Britain which has also been described on the continent of Europe, it has been shown by Bailey (1) in Canada and Mehta (11) in India that rust spores are carried considerable distances by wind. One would have thought it possible, in view of this evidence, that several of the forms in continental Europe would have occurred in Britain on account of wind dispersal. During the season when east winds prevail (February–March), however, *P. triticina* would not be generally prevalent in northern and central Europe. It is possible, however, that other forms common both to Britain and the continent of Europe are present in this country but have not been revealed in this investigation.

It is difficult to suggest an explanation for the number of new physiologic forms of *P. triticina* which have been shown to occur in England

and Wales. Scheibe (17) stated that in Germany forms increased in number and in inherent intensity of attack towards the east, indicating that the "specialisation centre" of *P. triticina* lies in eastern Europe (Russia and the Balkans). He indicated the possible significance of the prevalence of *P. triticina* on its aecidial hosts in Russia, where fourteen species of *Thalictrum* have been found to be susceptible to it. In connection with the production of new forms by hybridisation, Stakman, Levine, and Cotter (20) and Newton and Johnson (13) have demonstrated the importance of the part played by the barberry in the genesis of new physiologic forms of *P. graminis*. As no species of *Thalictrum* in this country has been found to be naturally infected by *Puccinia triticina* an explanation of the number of forms which are present here must be sought elsewhere. Johnston and Mains (8) state: "In the United States the native species of *Thalictrum*, as far as they have been tested, are resistant to *P. triticina*, and yet there are often many physiologic forms present in a single season. This is especially true of the eastern part of the United States. It seems, therefore, that some factor or factors other than the presence of the aecidial host must be sought to explain the distribution and abundance of physiologic forms of brown rust of wheat in this country."

It is suggested that mutation, which has been shown to be responsible for the origin of at least one physiologic form during the course of this survey, may be largely responsible for the creation of new forms in this country.

SUMMARY

1. Forty-six collections of *Puccinia triticina* have been tested for the determination of physiologic forms. The majority of these were studied as single-spore cultures. With the exception of three from Portugal the collections were made in various parts of England and Wales.

2. Ten new physiologic forms of *P. triticina* (Forms 66–75 inclusive) are described for the first time. Forms 66–73 were obtained from collections in Britain, and Forms 74 and 75 from Portugal.

3. A mutation in pathogenicity is here recorded for *P. triticina* for the first time, in the origin in the greenhouse of a variant of Form 10 from a culture of Form 66.

4. An apparently inherent instability in the behaviour of Form 66, as exhibited by an abrupt change in reaction on the variety Hussar from a resistant to a susceptible type, is discussed. This change cannot satisfactorily be correlated with environmental conditions or mixture of

cultures. Such instability was not observed in cultures of any other physiologic form encountered in this investigation.

5. Changes in environmental conditions affected the reactions of some of the differential varieties to certain physiologic forms. For example:

(*a*) Abnormal production of a type "x" reaction on certain of the differential varieties was found to be correlated in some forms with excessively high temperatures during incubation. Decrease in light intensity, combined with decrease in temperature, was found to be responsible for the production of an abnormal type "x" reaction by other forms.

(*b*) Increase in resistance in normally susceptible reactions was found in many forms to be associated with low light intensity and low temperature. Results, however, indicated that low light intensity seemed in general to be the more important factor in bringing about this reduction in susceptibility.

(*c*) Increase in susceptibility in the normally resistant reactions of certain varieties to some physiologic forms was found to be correlated with increased temperature and light intensity. Increase towards susceptibility of normally resistant reactions was not usually of a very profound type. Resistant reactions were found on the whole to be less sensitive to environmental fluctuations than susceptible reactions.

6. Infection of certain varieties, *e.g.* Malakof, Webster and Democrat, by *Erysiphe graminis* induced the development of pustules of a susceptible type by some physiologic forms of *Puccinia triticina* to which these varieties were normally resistant.

7. Form 66 appears to be the commonest and the most widely distributed form in Britain. This form was isolated fourteen times, Form 15 seven times, Form 67 five times, Form 68 three times, Form 70 twice, and Forms 10, 69, 71, 72 and 73 were each isolated once only.

8. Some variation in the annual distribution of physiologic forms was observed in the collections made near Cambridge, Newton Abbot, and Cardiff.

9. The fact that no previously described forms, except Forms 15 and 10, have been encountered is discussed. The apparent absence of an aecidial host for *P. triticina* in this country presents a problem as to the origin of the forms found here. It is suggested that mutation may account for the occurrence of some of these forms in view of the origin during the course of this investigation of a variant of Form 10 from a culture of Form 66, presumably by mutation.

This investigation was carried out at the suggestion and under the supervision of Mr F. T. Brooks. My sincere thanks are due to him for his helpful advice and criticism. Acknowledgments are also due to the University of the Witwatersrand for financial aid in the award of the H. B. Webb Gift Research Scholarship, which was held during 1932 and 1933.

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STUDIES IN BACTERIOSIS. XXII

I. THE ISOLATION OF A *BACTERIUM* ASSOCIATED WITH "FASCIATION" OF SWEET PEAS, "CAULIFLOWER" STRAWBERRY PLANTS AND "LEAFY GALL" OF VARIOUS PLANTS

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(With Plates X and XI)

Sweet peas affected by the disease known as "fasciation" usually produce a single upright stem with a development at the base of a mass of short swollen shoots and fleshy lumps (Plate X, fig. 1), but in severe cases the main shoot itself may fail to grow to any height (Plate X, fig. 2).

In 1927 Brown (1) concluded that "fasciation" of sweet peas was a form of crown gall caused by a weak or highly specialised strain of *Bacterium tumefaciens.* Muncie and Patel in 1930 (3) opposed this view, their experiments leading them to the opinion that the cause was physiological, namely the overcrowding of the roots of seedlings grown in small pots.

Shortly before the publication of Brown's paper the writer attempted to isolate *Bact. tumefaciens* from "fasciated" sweet peas, but without success, while the inoculation of known cultures of *Bact. tumefaciens* into sweet-pea seedlings resulted in the formation of typical crown galls at the point of inoculation, but in no case was there any "fasciation". An attempt was also made about this time to isolate *Bact. tumefaciens* from plants affected by the disease known as "cauliflower" of strawberries, but again only negative results were obtained. No further work was done on these diseases until the summer of 1933, when one severely fasciated sweet-pea plant was procured. This plant had one stem, 3 in. in length, considerably swollen at the base, and with no leaves unfolded; there were also numerous shorter, swollen stems about 1 in. long and a mass of fleshy lumps on the crown. Isolation plates were made from the basal lumps and all developed very mixed bacterial growth. Sweet-pea seeds, sterilised in mercuric chloride and germinated on blotting paper, were punctured by a needle in the hypocotyl region when the plumules were about $\frac{1}{2}$ in. long, and then planted five in a pot in small pots containing steamed soil. Two pots were kept as controls, two were inoculated with a water suspension of crushed tissue from the original plant, and the remaining four with suspensions of mixed bacterial growth from the isolation plates. Three weeks later each of the controls had the normal single stem, whereas, in all the inoculated pots, several of the seedlings had small basal shoots and slight swellings at the base of the main stem. A week later all the seedlings inoculated with bacterial cultures and three of those inoculated with tissue extract showed decided fasciation. After two months all but one of the plants inoculated with mixed bacterial cultures were severely fasciated, while of those inoculated with tissue extract three had rotted, three were fasciated and four were negative. All the controls had a single stem with no sign of basal swellings or growths.

In a second experiment all the seedlings inoculated with tissue extracts, or with mixed bacterial cultures isolated from a fasciated plant of the first experiment, produced the disease; in addition three seedlings grown in the soil from which this plant had been removed developed severe fasciation.

These two experiments having proved that fasciation was caused by an infective agent, a search was made for the causal organism. In this considerable difficulty was encountered, for while inoculations made from mixed bacterial cultures obtained from diseased material invariably gave positive results, numerous inoculations made from single colonies of various types failed to produce the disease. Unfortunately it was useless to reduce the numbers of extraneous organisms by sterilising the outside of the diseased shoots before making isolations, for, although this procedure markedly reduced the number of colonies on the plates, no inoculations from these were successful. All material from which isolations were made was therefore washed only in sterile water and, since the original inoculations were made with very mixed cultures, difficulty was caused by numerous types appearing again on reisolation from these. After a great number of single colonies had been tested with negative results it was thought that the disease might be caused, not by a specific pathogenic organism, but by chemical substances produced by mixed cultures of common soil species, the growth of which would be greatly accelerated by inoculation into sterilised soil. To test this hypothesis, isolations similar to those from the sweet-pea shoots were made from garden soil, and the resultant bacterial growth was inoculated into pots of sterile soil planted with sweet-pea seedlings; these all grew normally. In a further test a mixed culture from an isolation plate giving a strongly positive infection was subcultured, the subculture again subcultured, and so on five times. These five subcultures were tested on sweet-pea seedlings with completely negative results. The causal organism must therefore have been quickly crowded out by the more vigorous growth of the non-pathogenic bacteria. There remained the possibility that the disease was of virus origin, for the virus might be present in sufficient concentration in the suspensions of mixed growth made from isolation plates but not in the subcultures. To test this, badly fasciated shoots were crushed in water and half the suspension was passed through a bacterial filter. The two halves were then watered into the soil of pots planted with sweet-pea seeds. Fasciated plants were produced in the pots inoculated with the unfiltered extract but none in those inoculated with the bacterial-free filtrate. A similar result was obtained from the inoculations of extracts made from soil in contact with fasciated plants.

Having thus failed to demonstrate the presence of a virus, a further search for a pathogenic bacterium was instituted, and finally several virulent cultures were obtained. These cultures were from single colonies from isolation plates, but were of three different species. On further plating however a minute, very slow-growing colony of a distinct type was found on the plates made from virulent cultures. After further purification it was found on inoculation that these tiny colonies all produced fasciation, while negative results were obtained from the strains with which they had been mixed, all of which were of a markedly viscous nature, a fact which accounted for the difficulty experienced in separation. Several colonies of the organism thus isolated have been tested repeatedly by inoculation on sweet-pea seedlings and invariably produce severe fasciation (Plate X, fig. 3). The organism has been reisolated from the fasciated plants, and again proved virulent.

Isolations from "leafy galls"

In the type of growth called "Leafy Gall" a number of very short, hypertrophied shoots develop at the base of the plant or cutting, sometimes only just appearing above soil-level, but spreading horizontally till a large gall-like mass is produced, the older parts of which rot away but later become replaced by further growth. Photographs of such growths on chrysanthemum, carnation and *Schizanthus retusus* are shown on Plate X, fig. 4 and Plate XI, figs. 1 and 2. When a chrysanthemum plant is severely attacked, no normal basal shoots develop; in consequence when many plants of a particular variety are affected, the grower is unable to obtain sufficient cuttings to replenish his stock. The fasciation may develop in the propagating boxes a few weeks after the cuttings have been planted, a fact which suggests that infection may be carried by shoots taken from diseased plants. The disease is not so serious with carnations as the cuttings from these plants are made from the top growth, but a severely galled plant is much weakened, and the flowers may not be properly developed.

These growths are becoming increasingly common and are now causing concern in several nurseries.

On the suggestion of Prof. W. Brown, to whom the author is also indebted for procuring some of the material used in this work, isolations were made from "leafy galls" of carnations, chrysanthemums and a species of *Schizanthus*, from all of which bacterial strains agreeing closely in cultural characteristics with the sweet-pea organism were obtained. These strains were tested on sweet-pea seedlings and all produced typical "fasciation", the *Schizanthus* strain being particularly virulent.

ISOLATIONS FROM "CAULIFLOWER" OF STRAWBERRIES

The "cauliflower" disease of strawberry plants has been attributed to the nematode *Aphelenchoides fragariae*, but the connection has not been proved. Goodey (2), in a recent paper, says: "Another difficulty in the way of understanding the aetiology of both 'cauliflower' and 'red plant' in the strawberry is the fact that one and the same ectoparasite is found associated with two diverse conditions, the former giving rise to excessive bud formation and abnormal flowers, the latter on the other hand to blossom reduction or blindness. Again, if *A. fragariae* is the sole cause of 'cauliflower' and 'red plant' it is difficult to account for the fact that we may find a 'cauliflower' or 'red plant' crown side by side with a normal healthy crown on the same plant, the worms being present on both affected and healthy regions.... It will be evident from the foregoing remarks that still further investigations are required to elucidate the aetiology of these two diseases of strawberry plants. Meanwhile, *A. fragariae* cannot be dismissed as a possible or probable agent in their causation though the exact part played by it and the nature of its action remain to be determined." Goodey (2) gives the following description of the "cauliflower" disease:

"Affected crowns differ from healthy ones in that the stem and its appendages become much stunted and swollen almost to a fasciated condition. The surface of the crown is covered with a large number of dwarfed buds, thus resembling a 'cauliflower'. The inflorescence also becomes greatly affected and shows characteristic appearances....In extreme cases all the crowns of a plant may show the 'cauliflower' condition but it is not uncommon to find a plant showing one or two 'cauliflower' crowns as well as normal ones. The pathology of this condition has not been worked out."

In the summer of 1935 Mr W. Buddin, Advisory Mycologist of the Ministry of Agriculture, suggested that a search should be made for bacteria resembling the sweet-pea organism in strawberry plants affected by "cauliflower" disease, and sent a plant which he had inoculated some months previously with crushed "cauliflower" tissue and which showed typical symptoms of the disease. A second specimen was obtained from Mr D. O. Boyd of the Hants County Fruit Station. From both these plants strains of bacteria culturally similar to the sweet-pea organism were isolated and on inoculation both produced typical fasciation in sweet-pea seedlings.

INOCULATION EXPERIMENTS

With the exception of the preliminary experiments, in which the soil was steamed, and in other tests to find out whether soil sterilisation affected the development of the disease, the soil used in the inoculation experiments was sterilised in the autoclave for 4–5 hours at 20 lb. pressure, in small 3–4 in. pots. At first, sweet pea seed var. John Ingman was used, later a sample of mixed seed was tried and as this proved satisfactory, giving 100 per cent. fasciation of inoculated plants, its use was continued. The seeds were sterilised for 10 min. in 1/1000 mercuric chloride, washed in several changes of sterile water, and placed on sterile moist filter-paper in Petri dishes. When prick inoculations were made, the seeds were kept in the dishes until the plumule had emerged, and this was pricked with a very fine glass needle which in some tests was smeared with the culture. The seeds were planted three or four in each pot, placed under bell-jars in a greenhouse, and watered with sterile water. In the hot weather during July and August 1935 infection was apparent 8–10 days after inoculation and was very marked by the end of 3 weeks (see Plate X, fig. 3). Frequently in the tests made at this time the plumule failed to grow more than 2 in. or so in height and was often swollen and hypertrophied with no normal leaves, while a mass of secondary fasciated shoots developed round this primary shoot. All these growths originated at a point between the cotyledons and the primary shoot. In most cases inoculations of pure cultures of all the strains resulted in 100 per cent. positive results, but occasionally one plant in a pot of three or four would fail to show any sign of fasciation. It is possible that some varieties of seed may be less susceptible than others, but varietal tests have not yet been made.

After the various strains had all been proved virulent on prick inoculation, an experiment was made to find out whether wounding was necessary. The seeds were germinated as usual on filter-paper and half were pricked with a sterile needle before planting; the other half were not injured. A water suspension of the cultures was then poured into the soil surrounding the seeds. Fasciation developed equally well in both the pricked and uninjured seedlings with all the strains, thus proving that the organism was not dependent on the presence of wounds to produce its effect.

A further experiment was made to determine whether the age of the seedling at the time of inoculation was an important factor. Sweet-pea seedlings were inoculated with the sweet-pea strain both by prick and by unwounded surface inoculation, immediately after germination, and at 10, 17 and 24 days old respectively. Table I shows the result of this experiment.

Table I

Effect of age of sweet-pea seedlings on inoculation

No.	Age of seedling days	Type of inoculation	Plants fasciated %	Time taken to show disease days	Severity of attack
1*a*	Just germinated	Prick	100	8–11	All plants severely
1*b*	Just germinated	Surface	100	8–11	All plants severely
2*a*	10	Prick	100	17–22	Less severe than 1*a*
2*b*	10	Surface	75	14	Slight fasciation only
3*a*	17	Prick	75	15–25	As 2*a*
3*b*	17	Surface	0	—	—
4*a*	24	Prick	40	11	Slight fasciation only
4*b*	24	Surface	0	—	—

In each case rapid and severe fasciation developed in all the seedlings, both pricked and unwounded, which were inoculated just after germination. In the 10-day-old seedlings the disease took longer to develop and was less severe, especially in the unwounded seedlings, which only produced slight abnormal growth. The 17-day-old pricked seedlings were similar to the 10-day-old, while all the unwounded seedlings remained normal. By the 24th day seedlings had become still more resistant, only

40 per cent. of the pricked ones developing slight fasciation. Here again all the surface inoculated ones developed normally. Therefore seedlings must become infected at a very early stage in their growth for severe fasciation to result.

Prick inoculation of the aerial parts of the stems of sweet-pea seedlings gave negative results in every case with all the strains. Negative results were also obtained by the inoculation of the strains into tomato stems and on carrot slices, on both of which *Bact. tumefaciens* produced typical crown galls.

As it is impossible to ensure the sterility of plants grown in pots in a greenhouse, and in order to dispel any suspicion that infection might have been carried from one pot to another by insects, handling, watering, etc., the experiment was tried of growing inoculated sweet-pea seeds in sterile sand and water in small bottles and test-tubes plugged with cotton-wool. In every case seedlings inoculated with the strains from sweet pea, chrysanthemum, carnation, schizanthus and cauliflower-strawberry developed rapid and severe fasciation (Plate XI, fig. 3). As controls, seeds were left uninoculated, or infected with common soil and water organisms, namely *Bact. fluorescens liquefaciens*, *B. megatherium*, *B. mesentericus* and *B. coli*. Every control seedling developed a single shoot, with no signs of any basal growth or swelling.

These experiments afford conclusive evidence that the organisms isolated from "fasciated" sweet peas, "leafy galls" of chrysanthemum, carnation and *Schizanthus*, and "cauliflower" strawberry, are capable of producing the disease known as "fasciation" of sweet peas. Inoculations of the other host plants are now in progress, and much work remains to be done on the aetiology of the sweet-pea disease, the relationship of the organism to the host, and on the organism itself.

PRELIMINARY DESCRIPTION OF THE ORGANISM

The organism is a rod of very variable length, sometimes filamentous in old cultures, frequently found in groups forming **Y**'s, **W**'s, star-shapes, etc. Involution forms are common, the whole morphology of the organism being suggestive of *B. radicicola* or *Bact. tumefaciens*.

In old cultures intracellular bodies resembling spores are frequent, but when the malachite-green-safranin spore stain is used, these bodies stain deeply with the safranin and not with the malachite green.

The organism is Gram-positive and non-acid-fast. No motility has been observed in any medium, liquid or solid.

On first isolation, colonies on bouillon agar develop very slowly and remain small, but after several subcultures better growth is obtained. Colonies are round, raised, with entire margin, becoming dense, rather dry, sometimes papillate, sometimes with a narrow flat border; white at first, slowly becoming first pinky yellow, then deep yellow in old cultures. On bouillon agar slopes the growth is rather slow, somewhat viscid, white at first, becoming deep yellow with age. Growth in broth is feeble, mostly at the top, exhibiting marked aerobism; a laminate pellicle is formed. The optimum temperature for growth is 25° C.; no growth at 37° C. Thermal death-point is about 50° C. The organism does not liquefy gelatine or reduce nitrate, and has no diastatic action on starch. No acid or gas is produced in peptone water with the addition of glucose, lactose, saccharose, mannite, maltose or glycerine. In synthetic medium containing 1 per cent. of these substances, with brom-thymol blue as indicator, growth was meagre in all the cultures and there was no colour change until the seventh day, when the glucose and maltose tubes were slightly yellower than the control tubes. After 15 days, the carnation strain had turned the brom-thymol blue yellow in glucose, lactose, saccharose and maltose; the chrysanthemum and strawberry strains in glucose, lactose and maltose, and the *Schizanthus* and sweet-pea strains in glucose and maltose only. There is thus a slight difference between the strains in their growth in synthetic sugar media, but the production of acid is so very slow and slight that none can be said to be acid producers. *Bact. tumefaciens*, tested on the same media, had produced a yellow colour at the top of the tubes by the third day, and throughout the tubes on the sixth day, with the exception of the glycerine ones.

A further slight differentiation of the strains was noted in their growth in litmus milk. An alkaline reaction was developed in all, and the litmus was slowly reduced, but with considerably more rapidity by the carnation and *Schizanthus* strains than by the others. A soft clot developed very slowly in the carnation and sweet-pea cultures, followed by slow digestion; slow digestion without clotting occurred in the *Schizanthus* and one chrysanthemum culture, and no clot or digestion in another chrysanthemum and the strawberry cultures. This organism has not been identified with any known species.

SUMMARY

1. An organism has been isolated from fasciated sweet peas and found to reproduce the disease on inoculation into young seedlings.

2. Bacterial strains culturally similar to the sweet-pea organism have been isolated from "leafy galls" on chrysanthemums, carnations and

Schizanthus, and from "cauliflower" of strawberry. On inoculation all these strains produced marked fasciation in sweet-pea seedlings, and also produced fasciation of sweet-pea seedlings growing in inoculated sand cultures.

3. A preliminary description of the organism has been given.

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(1) Brown, N. (1927). Sweet pea fasciation. A form of crown gall. *Phytopathology*, xvii, 29–30.

(2) Goodey, T. (1935). *The Pathology and Aetiology of Plant Lesions caused by Parasitic Nematodes.* Imperial Bureau of Agricultural Parasitology Publication.

(3) Muncie, J. H. and Patel, M. K. (1930). Fasciation of sweet peas. *Amer. J. Bot.* xvii, 218–29.

EXPLANATION OF PLATES X AND XI

PLATE X

Fig. 1. Fasciated sweet pea with normal primary shoot. Result of inoculation with crushed fasciated tissue.

Fig. 2. Fasciated sweet pea. No normal shoots. Result of inoculation with a bacterium isolated from fasciated sweet peas.

Fig. 3. Sweet-pea seedlings 3 weeks after inoculation with the sweet-pea organism.

Fig. 4. "Leafy gall" of chrysanthemum.

PLATE XI

Fig. 1. "Leafy gall" of carnation.

Fig. 2. "Leafy gall" of *Schizanthus retusus*.

Fig. 3. Fasciated sweet-pea seedlings, grown in bottles in sand inoculated with bacterial strains isolated from various host plants.

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ADDENDUM

March, 1936. It has been brought to my notice that at the 26th Annual Meeting of the American Phytopathological Society, December 1934, P. E. Tilford reported that he had isolated a pathogenic organism from fasciated sweet peas. At the 27th Annual Meeting in January, 1936, he stated that he had isolated the same organism from fasciated growths on geranium and chrysanthemum. No description of the organism is given in the reports of these meetings. M. S. Lacey.

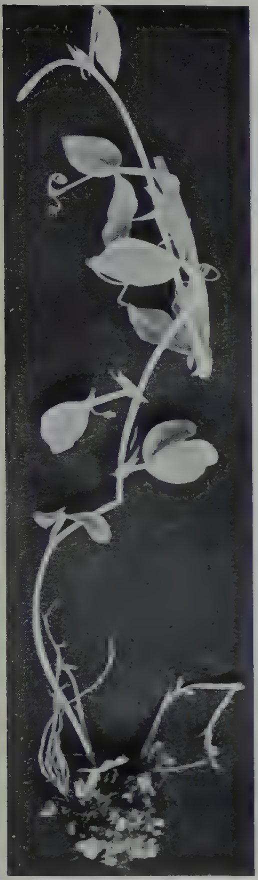

Fig. 1.

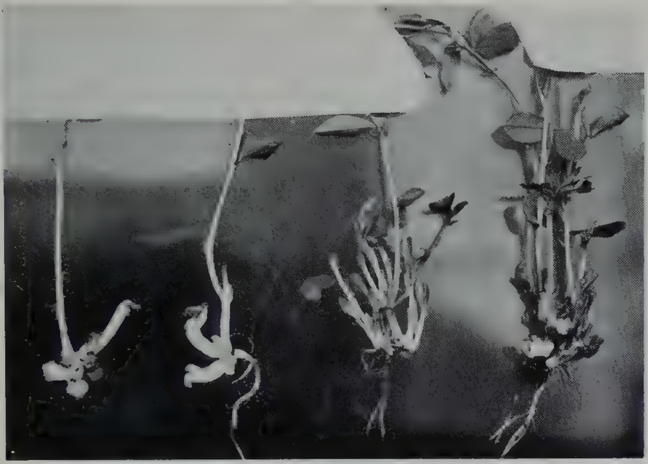

Fig. 3.

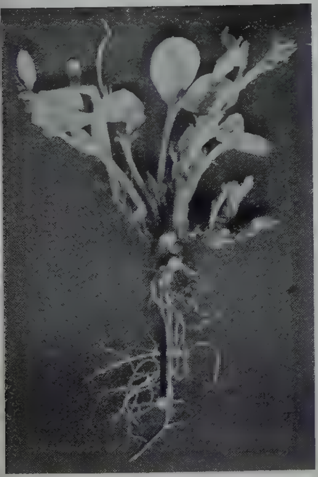

Fig. 2.

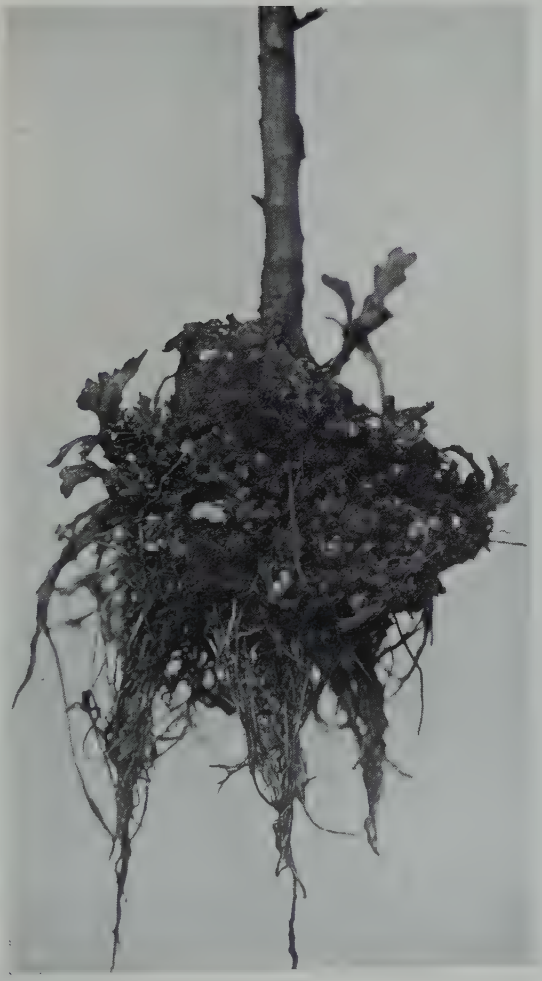

Fig. 4.

LACEY.—STUDIES IN BACTERIOSIS. XXII (pp. 302–310).

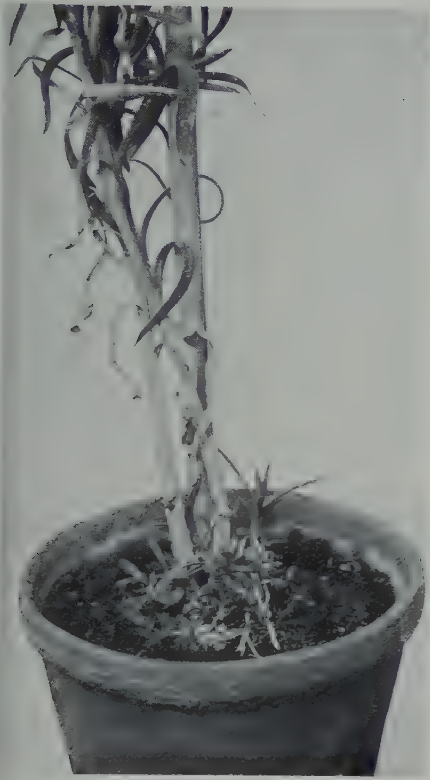

Fig. 1.

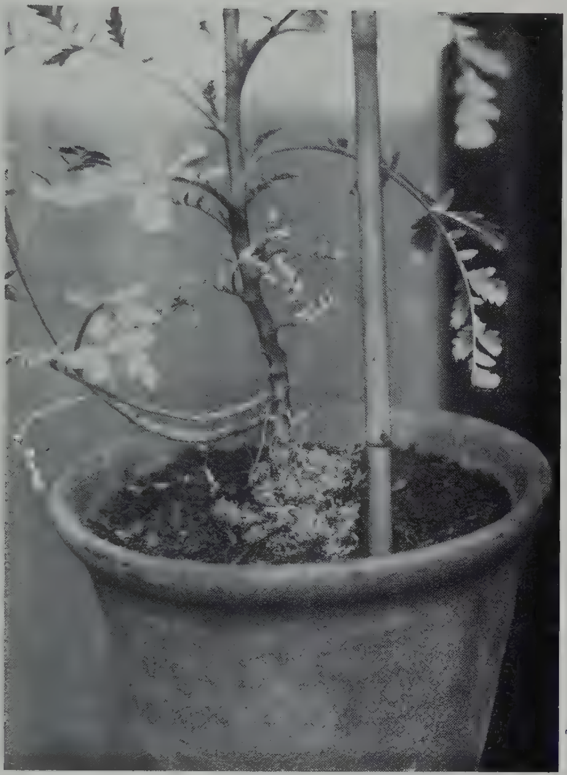

Fig. 2.

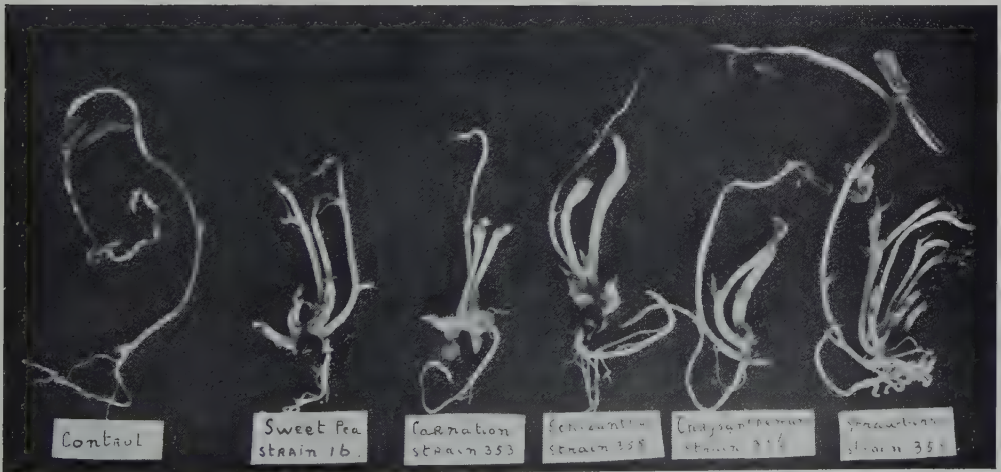
Control
Sweet Pea strain 16.
Carnation strain 353
Schizanthus strain 35
Chrysanthemum strain
Strawberry strain 35

Fig. 3.

LACEY.—Studies in Bacteriosis. XXII (pp. 302–310).

THE INSECTICIDAL PROPERTIES OF SOME EAST AFRICAN PLANTS

II. *MUNDULEA SUBEROSA* BENTH.

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(With 6 Text-figures)

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Introduction

Mundulea suberosa was first brought to the author's notice by Mr P. R. O. Bally, who collected a sample of the bark and forwarded it with a note to the effect that the natives on the coast preferred this plant to any other as a fish poison; it was, they stated, far more effective than *Tephrosia vogelii*. When tested in the laboratory it was found to be not only considerably more toxic to insects than *T. vogelii* but almost equal in effect to Derris root. Accordingly it was decided to conduct a thorough investigation of this plant.

The tree has been identified as *Mundulea suberosa* Benth., but some differences occur in the various specimens which have been accepted as *M. suberosa*, both in the Amani herbarium and at Kew, so that it is possible that there may be two varieties of this species; the one at present under examination having more pointed leaves and less corky bark than the other (as described in various reference books).

M. suberosa growing near the coast at Moa attains a height of 15–20 ft.; it grows well in the drier areas and is moderately common in the thin forest belts. When grown singly in the open it becomes somewhat bushy. When cut back it coppices freely.

It occurs to some extent in the Luengera Valley near Korogwe and in the Zanzibar islands, samples having been obtained from these two places; it is also reported from Mpwapwa and Arusha, but no samples have yet been obtained.

Young trees grown from Moa seed appear to be doing well in Amani, and it is hoped that a small plantation of these trees which has been started on the plains will provide material for investigation into the habits and the variability of toxicity, both seasonal and in individual trees, so that by selection it may be possible to produce trees of high toxicity.

HISTORICAL

Mundulea suberosa has been recognised as an efficient poison for a considerable number of years. The earliest reference available in Amani is for 1898 when Greshoff, in the Dutch Indies, carried out some experiments on its toxicity to goldfish (3,4). He found that extracts of the bark at a strength of 1 in 30,000 killed goldfish weighing 50 gm. in 15 min.; a further extraction of the same material with alcohol was toxic to the same extent when used at a strength of 1 in 4000, and a still further extraction with boiling alcohol at a strength of 1 in 1000; the residue was then non-toxic. Greshoff isolated a white powder from these alcohol extracts which he considered was probably rotenone (or derrid as it was then called); tested against goldfish he found that 1 part in 1 million stupefied the fish in 10 min., 1 part in 5 million in 40 min., and 1 part in 10 million in 50 min.

In 1925 Chevalier (1) reported that *M. suberosa* is an effective fish poison, and that it is cultivated for this purpose in the Sudan and Northern Nigeria. This author, however, appears to have mixed up *M. suberosa* and *Tephrosia vogelii*, because after giving a correct list of the various alternative botanical names and places of occurrence of *Mundulea suberosa*, as recognised in the reference books, he then goes on to describe a tree which is probably *Tephrosia vogelii*, certainly not *Mundulea suberosa*, stating that the leaves only are used as a fish poison and giving the usual method employed by natives for *Tephrosia vogelii*. The leaves of *Mundulea suberosa* are, however, not toxic. For this reason it is doubtful if the plant under cultivation in the Sudan and Northern Nigeria is *M. suberosa*; it is more likely to be *Tephrosia vogelii* which is known to occur there.

The plant is included in a list of fish poisons in a paper by Howes (5), where it is stated to be the source of the Indian fish poison "Soopli" or "Soopee".

An account of trials of *Mundulea suberosa* as an insecticide is given by Tattersfield and Gimingham (7), who tested two samples, one from South Africa which proved to have no appreciable toxicity to *Aphis rumicis*, and one from India which was quite toxic, the stems being the most active part of the plant. The authors concluded, however, that the results, although showing the plant to be toxic, did not suggest that it would take a very high place among fish poison plants as an insecticide.

A considerable part, at least, of the toxicity of this plant is, however, due to rotenone, and Fryer, Stenton, Tattersfield and Roach (2) have shown that *A. rumicis* is very resistant to Derris extracts and therefore to rotenone, so that it is possible that the sample tested by Tattersfield and Gimingham was more toxic to insects in general than they believed.

Subramaniam (6) also tested extracts of *Mundulea suberosa* and found them toxic to several different insects.

TOXICITY OF *MUNDULEA SUBEROSA* FROM DIFFERENT DISTRICTS

Preliminary tests having shown that only the bark and seeds were appreciably toxic, samples of bark were obtained from three districts: Moa, on the coast near Tunga, the Luengera valley near Korogwe and Prison Island, Zanzibar; seed was available only from Moa.

In Table I are shown the results obtained by spraying *Aphis tavaresi* del Guer. by the author's standard method. The bark was extracted with absolute alcohol to make a 10 per cent. extract, and this was diluted with 1 per cent. saponin to the required strengths. Ten insects were used (only the adult viviparous females being taken) in each test, and ten marks were allotted for each insect killed or in a completely moribund condition, five marks for each insect seriously affected and likely to die, and no marks for the remainder. Headings C (dead or moribund), B (seriously affected) and A (unaffected) respectively give the number of insects in each class and the last column the percentage marks allotted, 100 per cent. being equivalent to a complete kill.

The results in Table I show that the *Mundulea* bark from Moa is practically as toxic as Derris root with 5·4 per cent. rotenone content, but that the samples from Luengera and Zanzibar are less than half as toxic. Chemical determinations show that the latter contain approximately half as much rotenone as the Moa sample.

Work has therefore been concentrated on the Moa bark, and unless otherwise stated all results in this paper refer to bark from this locality.

As regards the various localities, Moa is at sea-level, the rainfall is low and the humidity comparatively low; Zanzibar is at sea-level, the rainfall much higher and the humidity high; whilst the Luengera valley is at about 1000 ft. altitude, the rainfall moderate and the humidity moderately high. It would thus appear that dry conditions favour the production of high toxicity.

Table I

Toxicity of Mundulea *bark from different localities*

Locality	Strength %	A	B	C	Marks %
Moa	½	—	—	10	100
	¼	—	1	9	95
	⅛	—	6	4	70
Luengera	1	—	1	9	95
	½	—	6	4	70
	¼	—	7	3	65
Zanzibar	1	—	1	9	95
	½	—	5	5	75
	¼	—	7	3	65
Derris root (5·4% rotenone)	½	—	—	10	100
	¼	—	1	9	95
	⅛	—	3	7	85

Observations will be made on the general conditions affecting growth and toxicity as opportunities occur.

TOXICITY OF DIFFERENT PARTS OF *MUNDULEA SUBEROSA*

All parts of the tree, including seed, were available at Moa, and it was therefore decided to test carefully every part of the plant.

Table II

Toxicity of different parts of Mundulea suberosa *to* Aphis tavaresi

Part of plant	Strength %	A	B	C	Marks %
Leaves	2	9	—	1	10
	1	10	—	—	0
Twigs	2	8	2	—	10
	1	9	1	—	5
Bark	½	—	—	10	100
	¼	—	1	9	95
	⅛	—	6	4	70
Roots	2	2	8	—	40
	1	5	3	2	35
Seeds	1	—	—	10	100
	½	—	2	8	90
	¼	—	5	5	75
Saponin alone	1	10	—	—	0
Nicotine	½	—	—	10	100
	¼	—	—	10	100
	⅛	—	2	8	90

Samples of the leaves, twigs, bark, roots and seeds were air-dried, finely ground and extracted in the cold with absolute alcohol as described in Part I of this series (8), thus yielding 10 per cent. extracts, *i.e.* 10 gm. of plant material extracted in 100 c.c. of alcohol. These extracts were diluted with 1 per cent. saponin solution in water to the various strengths to be tested. Table II shows the results which indicate that only the bark and the seeds are toxic to any appreciable extent, the former being comparable with nicotine. Further tests in the field confirmed these results.

TOXICITY OF *MUNDELEA SUBEROSA* BARK

The initial tests having shown the bark to be highly toxic to *Aphis tavaresi*, it was decided to carry out a thorough laboratory investigation of this material. The presence of rotenone was demonstrated by Durham's test, a red colour with a drop of strong nitric acid followed by a fugitive peacock-blue colour when a little strong ammonia solution is added, and accordingly in all toxicity tests on the bark a parallel series with Derris root was carried out for comparison. The rotenone content of the Derris was 5·4 per cent. on dry weight, and that of the *Mundulea* 0·9 per cent.

A. *Extracts*

(*a*) A large number of tests was made against *Aphis tavaresi*, each strength of spray being repeated twelve times; ten trials with nicotine

Table III

Toxicity of Mundulea suberosa *bark to* Aphis tavaresi

	Strength %					
Material	1/2	1/4	1/8	1/16	1/32	1/64
Mundulea bark	100	95	80	50	35	—
	100	100	60	55	40	—
	100	90	80	65	20	—
	100	90	80	80	40	—
	100	95	90	45	40	—
	100	95	80	45	35	—
	—	100	80	40	35	—
	—	100	70	55	25	—
	—	90	100	65	30	—
	—	95	70	55	40	—
	—	90	90	45	45	—
	—	100	80	55	35	—
(Mean)	100	95	80	55	35	—
Derris root (mean of 10)	100	97·5	87·5	77·5	65	30
Nicotine (mean of 10)	—	100	92·5	77·5	47·5	22·5

In 16 controls with 1 per cent. saponin the mean kill was 5 per cent.

and Derris were also made for comparison. In Table III, which gives the results obtained, the *Mundulea* bark extract was prepared as described above with absolute alcohol, the Derris was prepared in an exactly similar manner, and the nicotine was obtained by diluting a commercial sample of nicotine sulphate, the amount of actual nicotine base in solution being shown in the columns headed "Strength %"; 1 per cent. saponin solution in water was used in each case for diluting the extracts. In each trial ten insects were taken and marks were allotted as before, the figures given in the table being the percentage marks allotted, *i.e.* the calculated percentage kill.

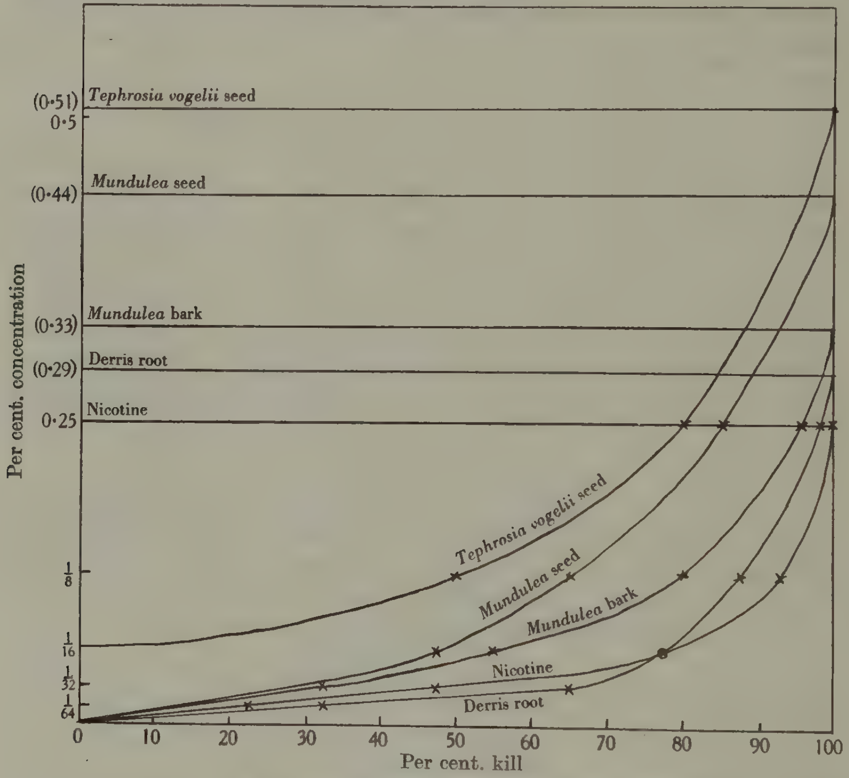

Fig. 1. Per cent. kill of *A. tavaresi* plotted against per cent. concentrations of toxic extracts. Horizontal lines show the concentrations required to give 100 per cent. kill.

The results are plotted in Fig. 1, which also shows the curves for *Mundulea* seeds (to be discussed later) and for *Tephrosia vogelii* seeds. It will be seen that Derris root and nicotine are nearly equal in toxicity over the whole range, nicotine being somewhat more toxic at higher concentrations; *Mundulea* bark is somewhat less toxic than Derris but not greatly so. The horizontal lines in Fig. 1 show the strength of each substance required to give just 100 per cent. kill, the values being nicotine 0·25 per cent., Derris 0·29 per cent. and *Mundulea* bark 0·33 per cent. The conclusion is that *Mundulea* bark is practically as toxic as Derris or nicotine to *Aphis tavaresi*.

(*b*) Another Aphid somewhat plentiful on coffee trees is *Toxoptera aurantii* Boy., which is a softer bodied insect than *Aphis tavaresi* and more easily killed. Against this insect *Mundulea* bark is slightly more toxic than Derris, as shown in Table IV. The results are means of duplicate tests on ten insects per test.

Table IV

Toxicity of Mundulea *bark to* Toxoptera aurantii

Material	%	A	B	C	Marks %
Control	—	9	1	—	5
Saponin	1	9	—	1	10
Mundulea	1/4	—	—	10	100
	1/8	—	—	10	100
	1/16	—	3	7	85
	1/32	—	6	4	70
Derris	1/4	—	—	10	100
	1/8	—	1	9	95
	1/16	—	5	5	75
	1/32	—	8	2	60
Nicotine	1/16	—	—	10	100
	1/32	—	2	8	90

These results are plotted in Fig. 2, and they show that nicotine is considerably more toxic to this insect and Derris slightly less so than *Mundulea* bark. The strength of each required to give 100 per cent. kill can be read off the curves as nicotine 0·06 per cent., *Mundulea* bark 0·125 per cent., Derris 0·15 per cent.

(*c*) *Caterpillars*. The most convenient method of testing the extracts on these insects is by dipping them into the solution, one at a time, for a definite number of seconds. *Brithys pancratii* Cyr., a very common caterpillar around Amani, was used, and each was dipped for 10 sec. Table V and Fig. 3 show the results obtained, the figures being the means of duplicate tests.

These results show that *Mundulea* bark and Derris are practically similar in toxicity to these insects, but that nicotine is much less so.

(*d*) *Lantana bug*, Orthezia insignis *Dougl.*, is a very common pest on many plants in Amani. It has a waxy covering and is therefore somewhat difficult to kill. Nicotine sprays have very little effect on it, but the

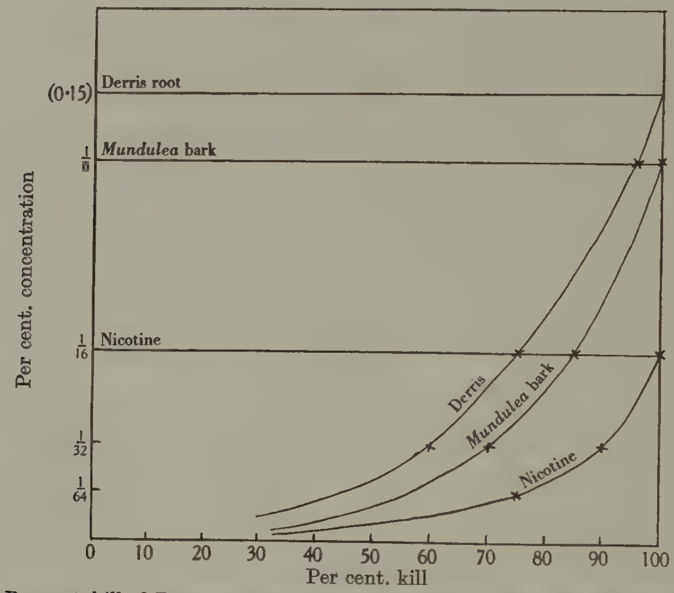

Fig. 2. Per cent. kill of *Toxoptera aurantii* plotted against per cent. concentration of toxic extracts. Horizontal lines show the concentrations required to give 100 per cent. kill.

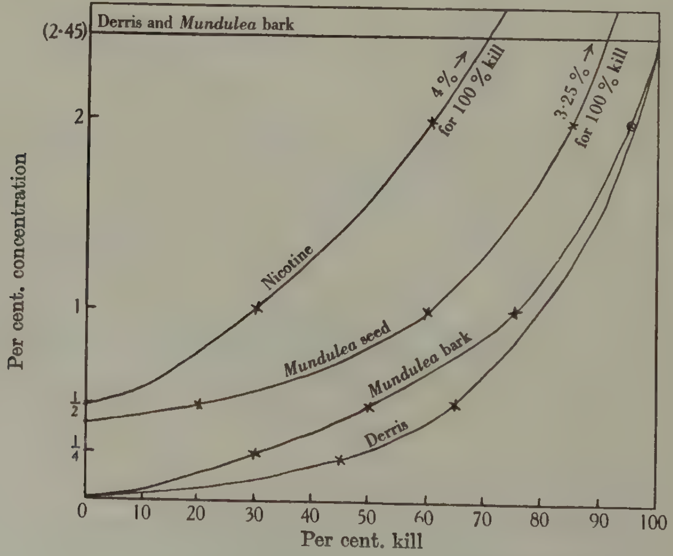

Fig. 3. Per cent. kill of *Brithys pancratii* plotted against per cent. concentrations of toxic extracts.

author has found both *Mundulea* bark and Derris extracts to be effective when used at about 2 per cent. strength and sprayed from only half the standard distance, *i.e.* 1 ft.

Table V

Toxicity of Mundulea suberosa *bark to* Brithys pancratii

Material	%	A	B	C	Marks %
Saponin	1	10	—	—	0
Mundulea	2	—	1	9	95
	1	—	5	5	75
	½	—	8	2	60
	¼	5	4	1	30
Derris	2	—	1	9	95
	1	—	5	5	75
	½	—	7	3	65
	¾	4	4	2	40
Nicotine	2	2	4	4	60
	1	4	6	—	30
	½	10	—	—	0

Table VI gives the results, using ten adult insects and spraying for 4 sec. from 1 ft. distance; figures are means of duplicates.

Table VI

Toxicity of Mundulea suberosa *bark to* Orthezia insignis

Material	%	A	B	C	Marks %
Saponin	1	8	2	0	10
Mundulea	2	—	—	10	100
	1	—	3	7	85
	½	—	6	4	70
	¼	3	4	3	50
Derris	2	—	—	10	100
	1	—	3	7	85
	½	—	6	4	70
	¼	3	4	3	50
Nicotine	4	4	4	2	40
	2	7	3	—	15
	1	10	—	—	0

The results show that *Mundulea* bark and Derris are identical in toxicity to *Orthezia insignis*, whereas nicotine has only a small killing power (see also Fig. 4).

(*e*) *Citrus Psylla.* A species of *Psylla* sometimes severely infects citrus trees in Amani, and *Mundulea* bark proves to be effective against this. The results of tests on the adult insects given in Table VII and Fig. 5 show that *Mundulea* bark and Derris are equally toxic to the insects although less so than nicotine.

(*f*) In Table VIII are given the concentrations of *Mundulea* bark, Derris root and nicotine required to produce just 100 per cent. kill of the

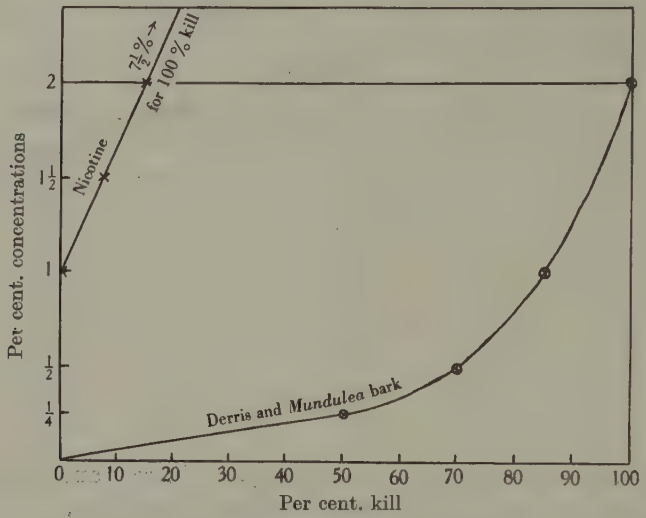

Fig. 4. Per cent. kill of *Orthezia insignis* plotted against per cent. concentrations of toxic extracts.

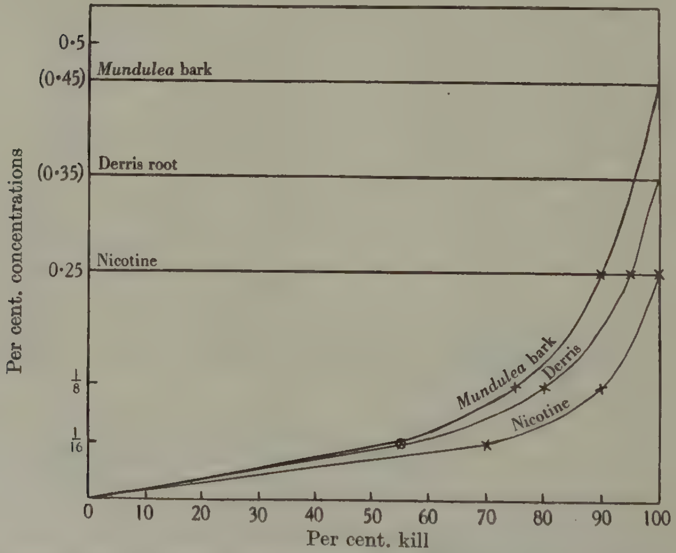

Fig. 4. Per cent. kill of *Citrus Psylla* plotted against per cent. concentrations of toxic extracts. Horizontal lines show the concentrations required to give 100 per cent. kill.

various insects used in the trials. These figures have been obtained from the curves plotted for each test, and they show quite clearly that *Mundulea* bark is for all practical purposes equal in toxicity to Derris root.

Table VII

Toxicity of Mundulea suberosa *bark to adult* Citrus psylla

Material	%	A	B	C	Marks %
Saponin	1	9	1	—	5
Mundulea	1/2	—	—	10	100
	1/4	—	2	8	90
	1/8	1	3	6	75
Derris	1/2	—	—	10	100
	1/4	—	1	9	95
	1/8	—	4	6	80
Nicotine	1/4	—	—	10	100
	1/8	—	2	8	90
	1/16	—	6	4	70

Table VIII

Concentrations required for 100 *per cent. kill*

Insect	*Mundulea* bark %	Derris root %	Nicotine %
Aphis tavaresi	0·33	0·29	0·25
Toxoptera aurantii	0·125	0·15	0·06
Citrus Psylla	0·45	0·35	0·25
Brithys pancratii	2·45	2·45	4·00
Orthezia insignis	2·00	2·00	7·50

B. *Dusting powders*

Air-dried *Mundulea* bark can be ground to a very fine powder, the bulk of it readily passing a 100 mesh sieve. Such powder has been used in a series of dusting experiments, comparing it with pyrethrum and Derris, against cockroaches.

Preliminary trials showed that pyrethrum powder knocked over the cockroaches in a few minutes, whereas no serious effects were noticeable with *Mundulea* and Derris for some hours; but at the end of 24 hours those dusted with the last two powders were all dead, whilst those dusted with pyrethrum were still kicking (on their backs) and did not die until between 2 and 3 days after dusting. Fresh, good-grade Kenya pyrethrum was used in all trials.

A dusting powder that has an immediate visible effect will tend to be preferred to one that has no apparent effect at the time, and in consequence slower acting powders are at a disadvantage commercially. The test shows that *Mundulea* bark and Derris powders are at least as effective as pyrethrum in killing household pests such as cockroaches and flies.

In order to correlate the effects of these powders with time it is necessary to devise some means of allotting marks according to the effect of the toxic substance on the insect. This is a more difficult problem than in the case of the spraying tests with aphides. After several trials a scheme of marking for cockroaches was devised which appears to give a reasonable numerical interpretation of the observed results.

Ten adult cockroaches were used in each test and marks were allotted as follows:

1, obviously disturbed and very active.
2, falling over but recovering actively.
4, on their backs frequently.
6, able to turn over with difficulty.
8, permanently on their backs.
9, kicking feebly.
10, dead.

With a little practice it was fairly easy to allot marks by this scale and thus to calculate the percentage "kill".

Ten cockroaches were placed in a glass dish, having a filter-paper inside it at the bottom, a glass cover was placed on top, and then 0·1 gm. of the finely ground powder was blown inside and the dish gently shaken at all angles so as to make sure that each insect came into good contact with the powder; the glass cover was then replaced by a wide-mesh gauze to allow air to the insects.

Marks were then allotted after various intervals of time. The figures given in Table IX are the combined results of four trials; many of the figures are the means of two or three results, some of them are single results. In Fig. 6 these results have been plotted; the numbers in heavy type in Table IX have been omitted from these curves as they are obviously erroneous. Figures for *Mundulea* seed are also included.

The curves show very clearly that pyrethrum is far more rapid in its action at first, but that as time goes on the cockroaches dusted with *Mundulea* bark and Derris are progressively more affected until after 20–22 hours the effect is equal in all three cases, about 90 per cent. kill. The last two substances finally produce 100 per cent. kill in about 29 hours, whereas pyrethrum takes about 50 hours to produce this result.

The initial effect of pyrethrum is very rapid, all the insects being seriously affected in a few minutes, and all being on their backs and unable to turn over again 20 min. after contact. Thereafter the action is very slow; the curve for pyrethrum in Fig. 6 shows this very clearly.

Table IX

Toxicity of powders to cockroaches

Time	Pyrethrum	Derris	*Mundulea* bark	*Mundulea* seed
10 min.	49	Nil	Nil	12
20 ,,	78	12	11	23
30 ,,	78	12	**20**	28
45 ,,	80	21	15	28
1 hour	84	19	21	32
3 hours	90	37	43	46
4 ,,	90	42	60	46
5 ,,	90	54	66	50
6 ,,	92	67	66	64
7 ,,	91	73	73	58
8 ,,	90	80	68	—
9 ,,	91	78	77	58
10 ,,	90	80	72	—
12 ,,	91	86	83	66
17 ,,	**98**	97	96	**98**
18 ,,	96	**100**	**100**	**100**
22 ,,	91	94	91	76
24 ,,	95	99	97	76
36 ,,	97	100	100	90
48 ,,	99	100	100	100

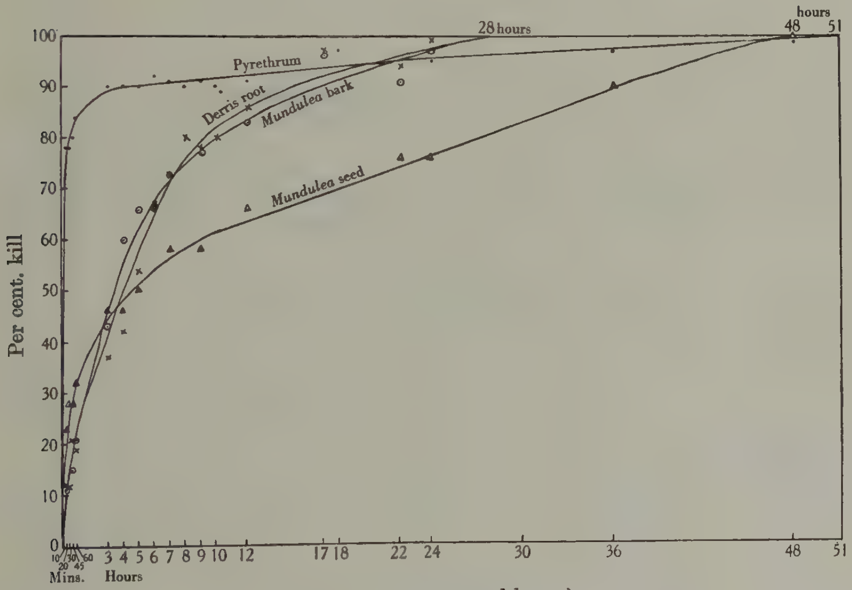

Fig. 6. Time after dusting with powders plotted against per cent. kill of cockroaches.

Mundulea bark and Derris are almost identical in action; the first effect is to cause the insects to become very agitated and make them dash rapidly about the dish for about 10–15 min., after which they become quiet with occasional violent movement; after several hours they commence to fall on their backs (although recovering again) during each period of activity, until after about 10 hours they are permanently on their backs. During the first few hours the cockroaches appear to be trying to clean themselves, especially the leg joints, with their mouths.

Further trials were carried out in which the cockroaches after dusting with the powders were removed to clean dishes, in which they were merely made to walk through the insecticide by putting a ring of powder around the inside edge of the dish and placing the cockroaches in the centre. In every case the results were similar to the above.

House-flies were also confined in glass dishes and shaken with the three powders; the initial effect was the same as with cockroaches, but those with pyrethrum were dead after about 8 hours and those with *Mundulea* and Derris after 10–12 hours. Tested against fleas on dogs the results were inconclusive; whereas the pyrethrum stunned the fleas rapidly, the *Mundulea* had no immediate effect other than to repel them and in consequence no dead fleas could be found.

C. *Paraffin sprays*

Cockroaches were sprayed with paraffin extracts of the three substances, prepared by shaking the finely ground powder with the paraffin in the correct quantity to produce 10 per cent. extracts. They were sprayed for 2 sec. from a distance of 2 ft. under the usual standard conditions. Table X shows the results. The cockroaches were examined

Table X

The toxicity of paraffin sprays to cockroaches

		Unaffected		Seriously affected		Dead		Kill %	
Spray	%	24 hrs.	48 hrs.	24 hrs.	48 hrs.	24 hrs.	48 hrs.	24 hrs.	48 hrs.
Paraffin	(*a*)	10	10	—	—	—	—	—	—
Pyrethrum	10	—	—	—	—	10	10	100	100
	5	—	—	2	2	8	8	90	90
	2½	—	6	7	1	3	3	65	35
Mundulea	10	—	—	—	—	10	10	100	100
	5	—	—	2	2	8	8	90	90
	2½	8	8	—	—	2	2	20	20
Derris	10	—	—	—	—	10	10	100	100
	5	—	1	1	—	9	9	90	90
	2½	4	8	5	—	1	2	35	20
Paraffin	(*b*)	10	10	—	—	—	—	—	—

after 24 and 48 hours, and it will be seen that all three extracts have identical toxicities at the higher concentrations, but that pyrethrum is slightly more toxic at 2½ per cent. strength, although most of these insects seriously affected after 24 hours had recovered in 48 hours.

Used as a house spray against flies 10 per cent. extracts of *Mundulea* bark had a strong repellent action but otherwise no apparent immediate effect; several hours later, however, dead flies could be picked up. 10 per cent. pyrethrum extracts bring down flies in a few minutes.

Extracts containing 7½ per cent. *Mundulea* and 2½ per cent. pyrethrum appeared to be as rapid in action and as effective as 10 per cent. pyrethrum, the presence of the latter producing the rapid stunning effect but the former being the chief cause of death.

TOXICITY OF *MUNDULEA SUBEROSA* SEEDS

In the preliminary results as shown in Table II extracts of the seeds were about half as toxic as bark extracts.

Only small quantities of seeds were available. The results of further trials against *Aphis taveresi* are shown in Fig. 1, from which it can be seen that 0·44 per cent. extracts are required for 100 per cent. kill as against 0·33 per cent. for *Mundulea* bark, 0·29 per cent. for Derris and 0·25 per cent. for nicotine.

Extracts of the seed were also tried against the caterpillar *Brithys pancratii* Cyr., and the results are shown in Fig. 3. The toxicities are again in about the same ratio, namely 3·25 for 100 per cent. kill with *Mundulea* seed and 2·45 per cent. with *Mundulea* bark and Derris.

The result of dusting the powdered seed on cockroaches is shown in Table IX and Fig. 6, where it will be seen that the initial effect is somewhat greater than with the bark and with Derris, but that after 3 hours it becomes slower in action and eventually takes 48 hours to produce 100 per cent. kill.

As a paraffin spray the seeds have little effect on cockroaches or flies.

All these tests indicate that *Mundulea* seeds are toxic, weight for weight, to an extent of about 75 per cent. of that of the bark. The comparatively small yield of seeds as compared with bark makes their use commercially unlikely.

AQUEOUS EXTRACTS

Attempts have been made to prepare direct water extracts, but in every case these have been found to be less toxic to *Aphis tavaresi* than alcoholic extracts. Extracts were made by shaking the powdered bark with cold water and leaving for 24 hours, with occasional shaking, before filtering; by shaking with cold water, filtering at once and spraying at once; by heating on a water-bath with water; and by shaking with water and not filtering; in every case 10 per cent. extracts were prepared, *i.e.* 10 gm. bark to 100 c.c. water.

It was not found possible to spray the unfiltered extract, as the nozzle was repeatedly choked by the solid particles. Table XI shows the results obtained for the other three water extracts and indicates that such extracts are considerably less toxic than alcoholic ones. In the case of cold-water extracts, prolonged contact with the ground bark appears to cause destruction of the toxic properties; this may be due to enzyme action, since heating or immediate use in the cold prevents this loss: this question is being further investigated.

Table XI

Toxicity of aqueous extracts of Mundulea *bark*

Extract	½ %	¼ %	⅛ %
Cold water, 24 hours	55	35	20
Cold water, at once	90	65	25
Hot water	90	75	45
Alcohol	100	95	80

In a preliminary test, freshly made suspensions of the finely ground bark in water were more toxic than alcoholic extracts to caterpillars dipped into the solutions, but suspensions 24 hours old were considerably less toxic. Further work on the stability of these suspensions is necessary and will be carried out in due course.

FIELD TESTS

It has not yet been found possible to carry out any large-scale trials in the field, but a number of tests on a small scale has been made and *Mundulea* bark extracts have been found very efficient against many insects *in situ*.

Twenty *Hibiscus* trees which were infested with aphides were sprayed with ¼ per cent. *Mundulea* extracts in 1 per cent. saponin; after 24 hours three leaves picked at random showed 5, 5, and 11 insects alive with 317, 263 and 401, respectively, dead; after 48 hours no living insects could be found on the trees. On an unsprayed tree 95 per cent. of the aphides were alive.

Two lemon trees infested with citrus aphis were sprayed with the same spray; after 24 hours there were, on two leaves picked at random, (1) 10 alive and 96 dead and (2) 7 alive and 81 dead; after 48 hours all were dead.

A bed of garden dahlias infested with a species of aphis was completely cleared of these insects by a ½ per cent. extract in ½ per cent. soft soap.

Hibiscus trees covered with Lantana bug (*Orthezia insignis* Dougl.) were sprayed with 2½ per cent. extracts of *Mundulea* bark in ½ per cent. soft soap on two consecutive days; 48 hours after the second spraying no living insects could be found on the trees. This spray is now used regularly in Amani gardens against Lantana bug which infests a number of different plants; this strength also kills any aphides or similar insects present at the same time.

Three citrus trees infested with Citrus *Psylla* were sprayed with 1 per cent. *Mundulea* bark in ½ per cent. soft soap; after 24 hours all adults and active nymphs were dead, whilst about 10 per cent. of the sedentary nymphs, which are protected by a waxy scale, were also dead, the remaining 90 per cent. being apparently unaffected. Three sprayings on alternate days were successful in killing all the insects and thus clearing the trees. The probable explanation of 10 per cent. of the sedentary nymphs being killed by one spraying is that they were still wholly or partially in the active stage when sprayed. By spraying three times on alternate days all nymphs were probably caught in the active stage.

Discussion

The results given in this paper together with some further information obtained at various times show that *Mundulea* bark obtained from Moa is appreciably more toxic than that obtained from other localities. Two possibilities suggest themselves; either the difference is due to climatic conditions, or the Moa trees may belong to a variety of the main species.

As pointed out, the climate at Moa is drier and hotter than in the other two localities from which samples were obtained, but on the other hand the plants from Moa differ in certain characteristics, such as narrower leaves and smoother bark, from the description of *M. suberosa.* Whilst the leaves from all three localities are narrower than those of most of the herbarium specimens, the bark of the Zanzibar and Luengera samples, especially the latter, is far more corky than the Moa ones and agrees well with the descriptions.

It is hoped to obtain samples from other districts for detailed examination.

In the meantime young trees reared from Moa seed have been planted out on the plains, and it is hoped in due course to obtain information on the question of rate of growth, best time for harvesting the bark and variation of toxicity in individual trees.

The chemical composition of the bark will be dealt with in a further paper, but it may be mentioned here that the rotenone content of the Moa bark is about 0·9 per cent. and of the other two about 0·5 per cent.

If the trees can be readily cultivated there seems no reason why the bark should not become of interest commercially.

SUMMARY

Mundulea bark from Moa district is as toxic to insects as Amani Derris root containing 5·4 per cent. rotenone. That obtained from two other districts is only about half as toxic. The former tree may be a variety of the ordinary *M. suberosa.*

The powder dusted on cockroaches and flies, although having a much smaller initial effect than pyrethrum and not producing any rapid knock-down, yet causes death in about half the time; Derris has the same action. The same remarks apply to paraffin extracts of these substances.

Mundulea seeds are about three-quarters as toxic as the bark, but are unlikely to be of any commercial value on account of their scarcity.

Experiments are in progress in the cultivation of *M. suberosa* from Moa seed, and it is hoped that the bark will find a commercial outlet.

I wish to record my thanks to Mr T. W. Kirkpatrick for entomological information, to Mr P. J. Greenway for botanical assistance and to Mr C. Mumford, manager of Moa estates, for collecting the bark which has been used in these experiments.

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OBSERVATIONS ON THE LIFE HISTORY AND CONTROL OF THE CABBAGE APHIS, *BREVICORYNE BRASSICAE* L.

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(With Plate XII)

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INTRODUCTION

THE cabbage aphis, *Brevicoryne brassicae* L., is a common pest wherever cabbage or related plants are grown. In this country the intensity of its attack varies from year to year, but it is often responsible for serious losses in crops of such plants as broccoli, Brussels sprouts, cabbage and cauliflowers. In the hot dry season of 1921 it was very numerous and exceedingly destructive, and also again in 1929.

In 1933 it caused such serious losses to market gardeners that they requested the Ministry of Agriculture to make arrangements for the special study of this pest as soon as possible, and, at the request of the Ministry, the writers commenced observations on the subject in November 1933.

HOST PLANTS

The most comprehensive list of food plants of *B. brassicae* (see below) is given by Börner (1). He states that attacks of this aphis were most serious upon cruciferous plants containing mustard oil, colonies being found on the leaves, flowers and fruits of such plants. Related plants with no mustard oil were only attacked when planted in close proximity to badly infested mustard oil-containing plants. Winged forms were found to alight on plants not containing mustard oil but not to feed upon them. The following is Börner's list:

Cruciferous plants definitely host plants of B. brassicae

Diplotaxis tenuifolia
D. muralis
Erucastrum pollichii
E. obtusangulum
Brassica nigra
B. oleracea
B. napus (all cultivated forms)
B. rapae (all cultivated forms)
Sinapis alba
S. arvensis
Eruca sativa
Raphanus raphanistrum
R. sativus (all cultivated forms)
Rapistrum rugosum
Myagrum perfoliatum
Crambe maritima

Cruciferous plants on which migratory colonies have been found

Bunias erucago
Sisymbrium officinale
S. sophia
Arabis (*Turritis*) *glabra*
Lunaria annua (seedlings)
Lepidium graminifolium
L. amplexicaule
Cochlearia arctica

Börner also gives a list of Cruciferous crops on which *B. brassicae* had not been found up to 1921.

The species has also been recorded on the following non-cruciferous plants:

Leguminosae: on vetch in West Oregon, U.S.A., by H. E. Ewing (3); on beans in U.S.A. by W. J. Zaumeyer (6).

Malvacae: on cotton in Turkestan by V. Plotnikov (4).

Resedaceae: on *Reseda luteola* in Germany by Börner (1).

Solanaceae: on tobacco in Poltara, Russia, by D. N. Borodin (2).

During 1933–5, the writers have found *B. brassicae* on the following cultivated plants:

Broccoli, Brussels sprouts, various kinds of cabbages, cauliflowers, marrow-stem kale, white mustard and swedes, and on the following weeds:

Charlock (*Sinapis arvensis*), shepherd's purse (*Capsella bursa-pastoris*) and *Raphanus maritimus* (near Bonchurch, Isle of Wight). Mr D. Boyes, the Director of the Horticultural Research Station, Cambridge, also found this aphis on *Cardamine hirsuta* in a greenhouse at the Horticultural Station on June 4, 1934, and on *Brassica* (*Diplotaxis*) *muralis* at Shippea Hill, Suffolk, on October 7, 1935.

SYMPTOMS OF ATTACK

Market gardeners are only too familiar with the mealy masses of "blight" that so frequently make their *brassicae* plants so unsightly.

Direct damage is caused principally by the withdrawal of sap and injury to the tissues. When present in large numbers the aphides markedly reduce the size of the leaves and cause them to wrinkle and curl; under dry conditions they may also cause wilting of the leaves. The part of the leaf where the insects are numerous often turns yellow while the rest of the leaf may remain green, though frequently the whole leaf turns yellow and sometimes leaves are killed. On seedling *brassicae*, the attacked plants are readily recognised by the curling of the infested leaves (see Plate XII, fig. 1). These plants are stunted and never do as well as similar plants which are not attacked. In older plants, the aphides occur most commonly on the older leaves and on Brussels sprouts, broccoli and cauliflowers are more abundant on the lower surface than on the upper surface. In addition to the direct damage to the leaves, serious loss is occasioned in the case of broccoli, Brussels sprouts and cauliflowers by the secretion of "honey-dew", which give these plants a dirty appearance and reduces their market value. The presence of aphis in the parts of the plants which are eaten greatly reduces their value and sometimes causes the housewife to reject them altogether. On Brussels sprouts, the lower sprouts usually suffer more than the upper ones; and later in the season it is often possible to pick good sprouts from plants which at the first picking produced no marketable crop.

Growers of *brassica* seed also suffer serious losses due to the sucking of these aphides. When present in large numbers (see Plate XII, fig. 2) they prevent the formation of seed in a considerable percentage of the "pods" and greatly hinder the development of others. When swedes are badly attacked, the leaves are stunted and wrinkled and the crop much reduced. No serious attacks were found on marrow-stem kale.

LIFE HISTORY

Theobald (5) gives the life history of *Brevicoryne brassicae* as follows: "It appears first of all in May, usually starting in small clusters beneath the leaves, and by June alatae appear and they fly far and wide. Several generations occur and they may be found on the plants right into December. In November the alate males and oviparous females appear and the latter lay their eggs on the winter greens. The black eggs often occur in great masses. The Stem Mother appears hatched from the winter eggs in

March. This form will live for over a month and at first reproduce very slowly. The adults derived from these live about the same time and as the summer goes on increase more and more rapidly; the greatest rate of increase is usually in September and October on into the early part of November. Sexual forms may occur as early as October, but most in November and December. The ova seem to be laid indiscriminately beneath the leaves and on the stems, now and then they may be found on the upper surfaces. Herrick has traced twenty-nine generations in the year. They also pass the winter in the adult stage, specimens sent me from Cheshire in 1911 at the end of November lived through the winter, out of doors. This has also been observed in America (Herrick, Webster, Quaintance, etc.)."

The writers' observations given below show that the life history varies from season to season, and may differ from the above account.

Observations on the life history in 1933–4

These observations were commenced on November 21, 1933, on fields of *brassicae* and cruciferous weeds in the market-garden areas of Bedfordshire, Cambridgeshire and Huntingdonshire. At this date a large number of aphides (a few of which were winged) and eggs were found in all districts on broccoli, Brussels sprouts, cabbage and cauliflower.

On November 27 a number of seed beds of autumn Brussels sprouts in Bedfordshire were examined, and in one of these a few live wingless aphides were found. On December 4 a few were also found in a similar seed bed in west Cambridgeshire. On February 12 no live aphides or eggs could be found on these plants.

Eggs at this time were very easy to handle, and could be picked off with a blunt needle without fear of being burst. After the first severe frosts, eggs became very fragile and the least touch with a needle burst them, several being destroyed before one could be removed successfully.

The following counts will indicate the numbers and distribution of the various stages of this aphis on broccoli plants. The outer leaves were the most heavily infested and the numbers fell off rapidly as the centre of the plants was approached.

November 21, 1933. *Two leaves of broccoli from near Histon, Cambs.*

Stage	Underside leaf	Upperside leaf	Total
Winged forms	8	3	11
Wingless	418	111	529
Parasitised	97	11	108
Eggs	19	6	25

December 4, 1933. *Two leaves of broccoli from Gamlingay, Cambs.*

Stage	Underside leaf	Upperside leaf	Total
Winged	8	4	12
Wingless	390	20	410
Parasitised	80	16	96
Eggs	150	17	167

The total number of wingless individuals on a broccoli plant with fourteen leaves, partly counted, partly estimated leaf by leaf, was nearly 2000.

The total number of eggs on the same plant was 1011, and of these 651 were on the leaves and 360 on the stem.

In January 1934, no winged aphides were found and the numbers of wingless forms rapidly decreased, the last being found on February 1. These (three in number) were on the *inside* leaves (2½ in. long) near the growing point of a broccoli plant. From that date no aphis (except an occasional *Myzus persicae*) was found on either old plants, seedlings, or cruciferous weeds. Nothing but masses of eggs remained.

On Brussels sprouts, the eggs were most abundant on the main stalk of the plant, a number were present on the stalks of the sprouts, and a small proportion were also found on the sprouts themselves and on the petioles and leaf blades of the large leaves of the plant. Some idea of the large numbers present may be obtained from Plate XII, fig. 3.

Eggs were also numerous on cabbage plants, but only a few were found on kale. No aphides or eggs were found on cruciferous weeds.

In the spring, aphides were found on April 10 on broccoli, Brussels sprouts, and cabbage which had been cut off and allowed to sprout. These were all small but some had already moulted. By the end of April, nearly all the fertile eggs had hatched. At this time the Brussels sprout plants had sent up flower heads, and the young insects were feeding on the unopened flower buds.

On April 10 small aphides were also found on shoots of sprout stalks in a dump on the side of a field near Biggleswade. A number of other dumps were examined and it was found that shoots were few in number and usually only produced on those plants whose roots were touching the ground. In some dumps no aphides were found and when present they were never numerous.

The examination of fields in which old stalks of Brussels sprouts had been ploughed in showed that a number of shoots were growing up from partly buried stalks. These shoots were usually free from aphis even when eggs were numerous on the stalks. Only two individuals were found on all the shoots examined.

In March 1934 a number of Brussels sprout stalks with many viable eggs on them were buried, some at a depth of about 4 in., others from 8 in. to 1 ft. Seedling sprouts were planted out later above the buried stalks. The plants were carefully examined on May 5 and some of the stalks were dug up. No viable eggs were found upon the stalks and no aphides were found upon the plants. In June and July the plants were still clean.

During the first three weeks in May, small colonies of aphides were found on the flowering stalks of broccoli, Brussels sprouts and cabbage. These were usually present among the tight unopened clusters of flower buds (only occasionally were they found on the leaves). Single aphides, or colonies of two or three were common, but colonies of six or seven were also occasionally found. The first viviparous young were found on May 6.

On May 24 about 2 acres of cabbage plants near Gamlingay were examined. This plot had been abandoned by a smallholder and the plants had formed flower heads which were swarming with aphides especially on the unopened flower buds and in masses on the stems just below (see Plate XII, fig. 2). *A winged form was found on this occasion, the first record for the season.* A count of the infected plants on this field was made on June 4, three rows being chosen at random; aphides were found on 94 plants out of 104. On July 12 most of these plants were heavily infested with wingless and winged forms. On June 7 young cabbage plants no more than 100 yards from this field were still clean, but on June 26 winged aphides and one large colony of wingless aphides were found on these young plants. On July 12 several large colonies were present, and at this date aphides were noted in all the *brassica* fields visited. Winged forms which had migrated were first found in two fields near Biggleswade on June 18.

These observations indicate that there was an interval between the appearance of the first winged forms and their migration to the young crop. The aphides on the young plants did not increase rapidly at first but by the middle of August large colonies were present in a number of fields. Individuals with wing pads were first seen on August 15 in a garden in Cambridge, and fully winged forms were seen in a field of Brussels sprouts on August 31. Some of these were surrounded by newly-born wingless young, and as many as six colonies were present on one large leaf. From the middle of August until mid-September the aphides increased enormously, and the attack in all the fields visited varied from bad to very bad. Heavy rain fell at the end of September and early

October. An examination of Brussels sprout fields on October 9 showed that in most fields the number of live aphides had been very much reduced, and the sprouts which were picked after this date were very much cleaner than was expected. At this date, many of the aphides had been eaten by Syrphid larvae and numbers of others had been parasitised.

In the neighbourhood of Langford, however, the insects were still numerous. On September 11 an extremely bad attack on swedes was observed near Langford, and here aphides were seen to be walking rapidly on the ground from plant to plant. The swedes were ploughed in shortly after.

In the autumn of 1934, eggs were first found on September 29. Far fewer eggs were laid in the winter of 1934–5 than during the previous winter, and in early February many of these had shrivelled. Everywhere the percentage of shrivelled eggs was high, and on some plants they far outnumbered the sound ones; on several occasions 70–75 per cent. of shrivelled eggs were noted. Aphides were not found in autumn Brussels sprout seed beds during the winter of 1934–5 as they were in the winter of 1933–4.

Observations in 1935

In January of 1935 far fewer eggs were present on the plants than in the previous year.

In 1934 no live aphides were found after February 1, but in 1935 they continued to live throughout February and persisted in small numbers for some time after eggs had hatched on February 28. On March 16 stalks were collected on which aphides were present among the florets, but no eggs could be found on these plants even after a careful examination in the laboratory. It seems probable that viviparous forms lived through the winter and continued to reproduce the following season.

It must be remembered that the winter of 1934–5 was an exceptionally mild one and that eggs had hatched as early as February 28, whereas the corresponding date in 1934 was April 10. In 1935 large colonies were present in the flower heads on May 10, whereas in 1934 only small colonies were noted at this date and large ones were not found until May 25.

The first winged aphides of the season were observed on May 25, a day later than that on which they were found in 1934. Aphides were first seen on the new crop on June 12 as compared with June 18 in 1934. In both years there was an interval between the production of winged forms and their arrival on the new crop.

The first winged forms produced on the new crops were found on July 15 in a field of spring cabbage near Potton where the insects were very numerous, whereas in 1934 they were not present till after the middle of August. By the end of the third week in August the aphides had increased enormously in a number of Brussels sprouts fields, and the attack still further increased during September.

The rains during September and early October were much heavier than in 1934, but although the rain in 1934 appeared to assist in reducing the numbers of aphides, it seemed to have little effect in 1935. In mid-October parasites were fairly abundant, and Syrphid larvae and especially those of *Syrphus luniger* Mg. had cleared the aphides from many of the leaves. Aphides were, however, sufficiently numerous to be causing serious losses in the market value of crops.

NATURAL ENEMIES

Predators. The larvae of Syrphid flies were commonly found in all districts. Early in October, both in 1934 and 1935, they had killed all the aphides on some leaves and a large proportion on others. A number of larvae collected in October 1935 were reared and these all proved to be *Syrphus luniger* Mg. This species appeared to be by far the most valuable of the predators. The Coccinellid beetles *Adalia bipunctata* L. and *Coccinella 7-punctata* L. and their larvae were also common, but did not appear to be sufficiently numerous to be an important factor in reducing the aphides at any stage.

Parasites. Hymenopterous parasites were active from April to October (see Plate XII, fig. 4). Neither spraying with 0·05 per cent. nicotine nor dusting with 3 per cent. nicotine dust prevented the emergence of these parasites.

The following were bred out and later identified by Dr C. Ferrière.

Parasites:

Family Aphidiidae: *Diaretes rapae* Curtis.
Family Pteromalidae: *Asaphes vulgaris* Walk.
Family Calliceratidae: *Lygacerus testacimanus* Kieff.

The first two were by far the most numerous.

Hyperparasite:

Family Cynipidae: *Charips victrix* West. var. *infuscatus* Kieff.

Although these parasites were active during the summer, they were unable to prevent the aphis from multiplying rapidly when weather conditions were favourable. In August and September of 1934 and 1935 the aphides increased rapidly in numbers, and although in October parasitised aphides were numerous, the parasites did not appear to be such an important factor in reducing the number of aphides as did the larvae of the hover fly, *Syrphus luniger*.

CONTROL MEASURES

From the above observations it would appear that in the market-garden areas investigated, *Brevicoryne brassicae* lives through the winter mainly on *brassica* plants which are grown for seed. The chief method of over-wintering is by means of eggs on old plants, and this appeared to be the only method in the winter of 1933–4, though the writers' observations strongly suggest that adults lived through the following winter into the summer of 1935. Whether they over-wintered as eggs or adults, the main source of infection for the following summer was Brussels sprouts plants grown for seed. Other *brassica* seed plants also provide sources of infection, as do broccoli and cabbages which have been allowed to seed or cabbage plants which have been cut off in the spring and allowed to shoot again. Other less fertile sources are dumps of Brussels sprouts stalks and stalks which have been ploughed in and not completely covered, and further possible sources are autumn-sown Brussels sprout plants and other *brassicae*. No evidence was obtained that weeds carry the infection from one year to the next.

These observations suggest that control measures should be aimed at preventing the aphides from passing from old plants to the newly planted ones, as the acreage of the former is comparatively small.

Experiments in 1934

(1) On eight rows of Brussels sprouts seed plants near Langford. On May 11 four rows (31 yards long) were dusted with 5 lb. of 3 per cent. nicotine dust. On May 15 five live aphides were found on the untreated plot but none were found on the dusted plot.

(2) On seven rows of Brussels sprouts seed plants near Langford. On May 11 four rows (16 yards long) were dusted with 2¾ lb. of Derris dust (rotenone content 0·2 per cent.). On May 15 fifteen live aphides were found on the control plot and twelve live ones on the dusted plot.

(3) On one row of Brussels sprouts seed plants near Wrestlingworth, where very many colonies were present. On May 24 the whole row was dusted with 12 lb. of nicotine dust. This appeared to kill all the aphides except one colony which persisted until July 12.

(4) On a large plot of cabbage plants which had been allowed to run to seed near Gamlingay and were badly attacked: (*a*) On May 26 twenty-

nine plants were dusted with 3 per cent. nicotine dust. Examination on May 27 and 28 showed that some aphides were still alive, and on June 4 only two plants out of twenty-nine were free from aphides.

(*b*) On May 29 ten plants were *thoroughly* sprayed with 0·05 per cent. nicotine and soft soap. This appeared to kill all the aphides, but a careful examination on June 4 showed that, although nine of the plants were clean, two or three small colonies were present on one plant. Seven of these plants were still free from aphis on July 17.

(*c*) Ten plants were dusted on May 29, and although a large number of the insects were killed, some were found alive on May 30 and 31.

Neither spraying nor dusting killed the parasites within the bodies of parasitised individuals.

These experiments show that it is very difficult to get a complete control of this pest on *brassica* seed plants, but that thorough spraying with nicotine and soft soap gives a large measure of control, and also that thorough dusting with 3 per cent. nicotine dust kills a fairly large percentage of the insects.

(*d*) As most of the aphides on Brussels sprouts seed plants appeared to be present at the tips of the flowering stalks, it was thought that it might be an economic measure to remove these by hand on small plantings of seed plants. An attempt was made to do this on seventeen Brussels sprout plants grown for seed near Sutton (Beds). On May 25 three large colonies and a number of small colonies were removed. No winged forms were present at this date. On June 6 forty-one colonies and sixteen individuals (one winged) were found and removed. No parasites were seen. On June 12 one hundred and thirty-two colonies and twenty-five individuals (three winged) were found and removed. One parasite was seen. On June 17 two hundred and thirteen colonies and eleven individuals (three winged) were found and removed. No parasites were seen. On June 26 one hundred and forty colonies and one single aphis were found.

It seems probable that the colonies found from June 12 onwards had arisen from single aphides which had been missed at previous examinations. This method and probably also any method which aims at killing the colonies only does not appear to be satisfactory.

Later in the season experiments were carried out in a field of Brussels sprouts near Tadlow in order to determine the value of dusting and spraying large Brussels sprouts plants on which the aphis attack was fairly bad. 3 per cent. nicotine dust was applied at the rate of 35 and 43 lb. per acre. Some of these plants were also sprayed with nicotine at a strength of 3 oz. to 40 gallons of water, together with a spreader. These treatments were chiefly effective where large colonies were exposed, but none killed 50 per cent. of the aphides.

Recommendations

From experiments and observations, the writers suggest that control measures should aim at killing the aphides on seed plants before the winged forms leave them for the young crops.

(1) *Brassica* seed plants (*i.e.* those from which seed will be harvested) should be either (*a*) thoroughly sprayed during the second or third week in May (*i.e.* before any winged aphides are formed) with the following wash:

Nicotine (96–98 per cent.)	3 oz.
Soft soap	4 lb. or any suitable wetter.
Water	40 gallons.

or (*b*) thoroughly dusted with 3 per cent. nicotine dust during the second or third week in May. A second spraying or dusting will be necessary if colonies are found on the flower heads after the first treatment.

If all growers in market-garden areas where *brassica* crops are raised frequently would refrain from growing seed crops or grow them in other districts and would carry out the measures suggested below, it is probable that the aphis attack would be very much reduced.

At present we have little information on the distances which these insects travel or can be transported, but the writers' observations show that fields several miles from seed plants may suffer badly from this pest.

(2) All old Brussels sprouts, broccoli, cabbage and other *brassica* plants should be (*a*) ploughed under not later than May 15, steps also being taken to destroy any shoots which form on partly buried stalks, although this is not a frequent source of infection, or (*b*) the stumps of these plants should be put into heaps. These heaps should either be burned before May 15, or any stumps which have formed shoots should be moved and put on the top of the heaps so that the shoots may wither. The stumps which form shoots are usually those at the bottom of the heap which have rooted into the soil below.

(3) Autumn Brussels sprouts plants or other autumn-sown *brassica* plants should be carefully examined for the presence of this aphis at the time of planting, and if it is found the plants should be dipped before planting in a mixture of

Nicotine plus a wetter	3 oz.
Water	40 gallons.

(4) Experiments and observations show that this pest is difficult to

control on ordinary field crops, especially in August and September when the plants are large.

The infection of the new crops starts about mid-June, and the crop should be carefully examined from this time onwards. It is suggested that crops that are infected should be thoroughly dusted with 3 per cent. nicotine dust early in July, using 40 lb. of dust per acre or more, according to the size of the plants. A second dusting will probably be necessary later in the month.

We have not sufficient information to say whether dusting in August or September is an economic proposition (see p. 339).

(5) In Brussels sprout fields it is common to find occasional isolated plants which are smothered with aphis. These should be carefully lifted, put into bags, removed and destroyed.

SUMMARY

1. A study has been made of the life history of *Brevicoryne brassicae* L. in the market-garden areas of Bedfordshire, Cambridgeshire and Huntingdonshire from November 1933 to October 1935.

2. There was a variation in the behaviour of this pest in the two years. In 1934 the aphis over-wintered only as eggs on cultivated cruciferous crops (and particularly Brussels sprouts), whereas observations strongly suggest that in 1935 it over-wintered both as viviparous females and eggs. The hatching of the eggs was much later in 1934 than in 1935, but the first winged forms were produced at about the same date.

3. Predators and parasites were fairly abundant but not sufficiently so as to prevent serious damage.

4. It is suggested that control measures should aim at preventing the aphides from passing from old plants to newly planted ones. Nicotine sprays or nicotine dusts are suitable for this purpose. The pest is difficult to control on ordinary field crops.

The writers wish to acknowledge their indebtedness to Dr I. Thomas, of the School of Agriculture, Cambridge, and Mr J. W. Dallas, Agricultural Organiser for Bedfordshire, for their interest and help in these observations and experiments.

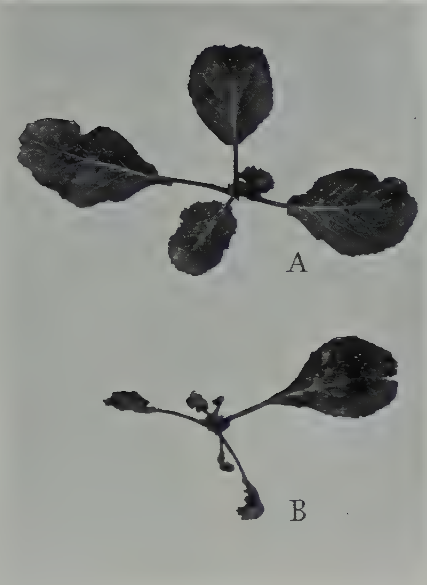

Fig. 1.

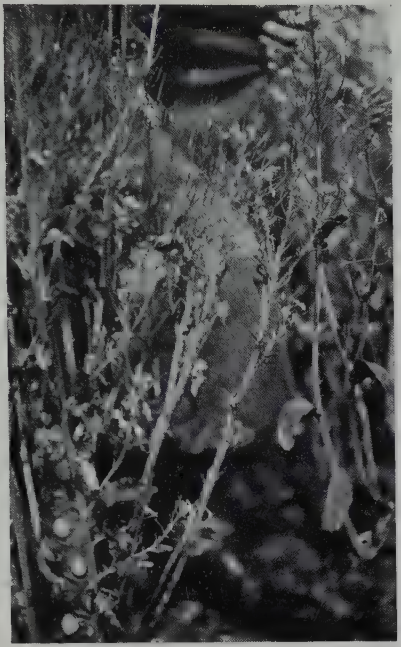

Fig. 2.

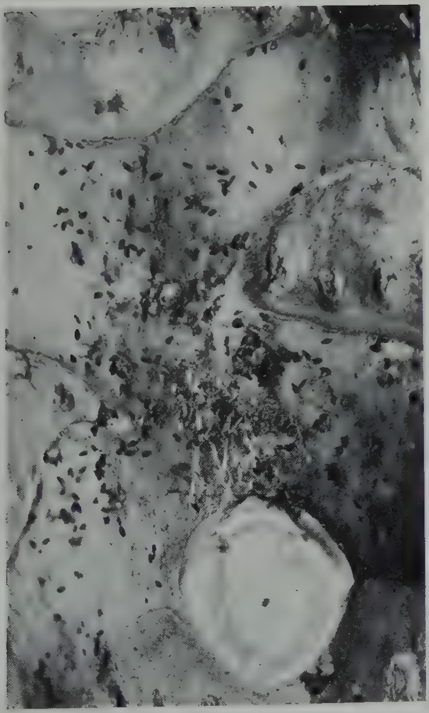

Fig. 3.

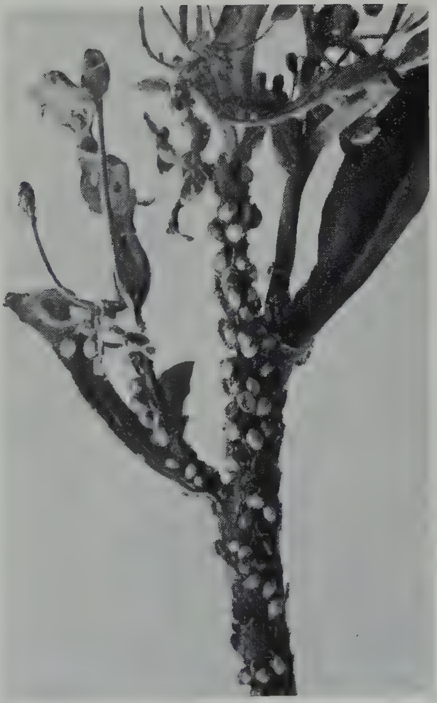

Fig. 4.

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EXPLANATION OF PLATE XII

Fig. 1. Young pickling cabbage plants sown in August. *A*, healthy; *B*, attacked by *B. brassicae*.

Fig. 2. Colonies of *B. brassicae* on Brussels sprouts seeds plants. Photo, June 7, 1935.

Fig. 3. Eggs of *B. brassicae* on the stalk of an old Brussels sprout.

Fig. 4. Flowering stalk of a cabbage plant showing parasitised aphides.

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THE BIOLOGY OF *LEPTOBYRSA RHODODENDRI* HORVATH (HEMIPTERA, TINGITIDAE), THE RHODODENDRON LACEBUG

I. INTRODUCTION, BIONOMICS AND LIFE HISTORY

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(With Plates XIII–XV and 4 Text-figures)

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INTRODUCTION

THERE are three closely related members of the Tingitidae which infest rhododendrons and azaleas chiefly in Europe and the United States of America. They are *Leptobyrsa rhododendri* Horvath (= *Stephanitis rhododendri* Horvath = *Leptobyrsa explanata* Heidemann) (21, 23), *Stephanitis pyrioides* Scott (= *azaleae* Horvath) (24, 44) and *S. oberti* Kol. (29). The present paper deals with *Leptobyrsa rhododendri* Horv., the only one, of the three above-mentioned species, known to occur in Great Britain, although it is not considered an indigenous species.

In the literature dealing with *L. rhododendri* a good deal of inconsistency exists concerning the name. According to van Duzee (59), the first mention of the species was made by Provancher (39) in 1887 who wrongly identified it as *Leptostyla oblonga* Say. Provancher's material has not been examined, but from his description and figure there is little to indicate that he was dealing with *Leptobyrsa rhododendri* Horv.

In 1905 Horvath described the insect for the first time as a new species, *Stephanitis rhododendri* (23), and in 1908 Heidemann (21) redescribed it, renaming it *Leptobyrsa explanata*; van Duzee (59) in 1917 synonymised *L. explanata* Heid. and *Stephanitis rhododendri* Horv., and there seems to be no doubt that they really are one species. The specific name, *rhododendri*, therefore has undoubted priority, although this is not necessarily true for the generic name. It seems that *S. rhododendri* Horv. is more correctly placed in the genus *Leptobyrsa* than *Stephanitis* (38, 63), although until specimens are compared with the genotype of *Leptobyrsa* (*L. steinii* Stål (47), which, incidentally, van Duzee does not mention) and with the other species of both genera, this cannot be decided with finality.

The earliest known record of *Leptobyrsa rhododendri* Horv. was from America (Pennsylvania) in 1877 (21), but it was apparently not recognised as a pest in America until 1906, when it was found in abundance in several localities in New York (9). Since then it has been constantly recorded as causing serious damage in many parts of the United States (see the numerous American references) and in British Columbia (13). The insect has also been observed, for the first time in 1923, in the Union of South Africa (32).

Ritzema Bos (4) seems to have been the first recorder of the pest on the Continent of Europe, for in 1905 he wrote of a Tingitid, taken at Boskoop in Holland, which resembled *Tingis oberti* and *T. chlorophani*. In 1907 (5) he again recorded it on rhododendrons from the same locality and stated that it had been known for four or five years in Holland. Dr T. Schoevers, of the Phytopathological Service, Wageningen, however, writes (*in lit.*) that it is now stated that the insect had been imported into Holland fifteen years before Ritzema Bos came to know of its presence there. Although Horvath's original material was from Boskoop the insect was referred to as *T. rhododendri* until 1915 in the reports of Ritzema Bos (7).

Subsequent to these records from Holland, the insect has been found in other European countries. Tullgren (56) and van Poeteren (60) refer to the insect in Belgium. Fron (16) and Marchal (33) in 1917 wrote of the pest occurring near Paris and Orleans on plants which had been imported from Holland three or four years previously and, later, Marchal and Foex (34) stated that the insect was a grave menace to the large horticultural establishments near Paris.

In Germany the earliest record appears to be 1915 (49). Schmidt (42), however, writes of the presence of *Leptobyrsa rhododendri* in Berlin (probably in 1927) and states that until 1927 *Stephanitis oberti* Kol. was the only insect recorded as damaging rhododendrons in Germany. Stichel (50) also did not consider *Leptobyrsa rhododendri* to occur in Germany in 1926. Schmidt points out that the literature on *Stephanitis oberti* in Germany probably deals with *Leptobyrsa rhododendri*, the two species having been confused. The extent of this confusion is not known, but it was thought by Lindinger (31) as late as 1927 that *L. rhododendri* Horv. *Stephanitis pyrioides* Scott and *S. oberti* Kol. should be regarded as synonymous. Stichel (51) refuted this, and in a paper by Laubert and Trappmann in 1929 (30) dealing mainly with *S. oberti*, figures of *Leptobyrsa rhododendri* were given from Crosby and Hadley (9) without an adequate legend to indicate which species they were intended to represent. *Stephanitis oberti* is known to occur in several localities in Germany, and Kaven (28) considered it to have been introduced there in 1911. *Leptobyrsa rhododendri* Horv. was noticed in Austria in 1927 (31).

In Great Britain it was generally believed that the first records of *L. rhododendri* were in 1910 from Fulham (12) and in 1911 from Kew (54), Harding (19), however, observed the pest in 1906 in Surrey, and Fox-Wilson (15) has pointed out that Raffill (40) observed it as early as 1901 at Kingston-on-Thames; this last is the earliest known record for Great Britain. The insect has been placed upon the list of insects scheduled in the Sale of Diseased Plants Order of 1927 (48).

II. THE ORIGINAL HOME AND SUBSEQUENT DISPERSAL

Leptobyrsa rhododendri Horvath is now present in many countries and the source from which it spread is largely a matter for conjecture. There are three hypotheses concerning the original home of the insect. The first is that Japan was the source; such an hypothesis was never, so far as the present writer knows, definitely put forward, neither can any reliable record be found of the insect occurring in Japan. Several writers (13, 14, 56, 58) have stated that it is found there, but no authority seems ever to have been given. Moreover, the American *Lists of Intercepted Plant Pests* (57) give no indication of the insect being imported, since 1923, into the U.S.A. from any country. It is the opinion of the present writer that the idea of the Japanese origin has no scientific foundation and that it arose in the following way.

Although Ritzema Bos himself did not know the country of origin, his writings imply, through the constant use of the terms "Japansche luis" or "Japansche wants", terms which he found in use in 1905 (Schoevers *in lit.*), that Japan was the original home of the insect. Two other Tingitids, *Stephanitis pyrioides* Scott and *S. oberti* Kol., the former a pest of azaleas, the latter of rhododendrons, are both present in Japan and (at least *S. pyrioides*) in Holland (6, 24, 28, 44, 61)[1]. It seems probable, therefore, that either one or both species may have become confused at an early date with *Leptobyrsa rhododendri* Horv. It is unlikely that Ritzema Bos himself confused them, at least later, since in 1910 (6) he referred to the two species separately in the same paper, but nurserymen in Holland may easily have failed to notice that more than one species existed and may have applied the term "Japansche luis" or "Japansche wants" to any or all of them. The confusion is apparent elsewhere, for Müller-Thurgau and others (37) used the names *pyrioides* and *rhododendri* synonymously and gave no author for either species. They also stated that the original home of "*Stephanitis pyrioides* (*rhododendri*)" was thought to be Japan. Millais (36) also considered it probable that two species existed, one called the "Catawbiense fly" in eastern North America and the other called the "Japanese fly" by Europeans in Japan. It is suggested by the present writer that the former name may apply to *Leptobyrsa rhododendri* Horv. and the latter to either *Stephanitis pyrioides* Scott or *S. oberti* Kol. Schoevers suggests (*in lit.*) that growers in Holland may have called the insect by the name "Japansche luis" on account of its exotic appearance or perhaps by confusing America and Japan!

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[1] *Tingis pyrioides* Matsumura is synonymous with *Stephanitis ambigua* Horvath (24).

The second hypothesis, put forward by Distant (12), was that India—"the home of wild rhododendrons" and of four species of *Stephanitis*—was the original locality; this view is supported by Sorauer (46). Millais (36), however, has pointed out that the probability of the insect having been imported from India to England on living rhododendrons is small, since scarcely without exception, all Indian rhododendrons have reached England in the form of seed.

The third and most likely hypothesis (8, 62) is that *Leptobyrsa rhododendri* Horv. is indigenous to America. Support for this view is given by the following facts, viz.: (1) the insect is extremely widespread there and feeds in large numbers on *Rhododendron maximum*, considered to be a species of rhododendron indigenous to America (1), and on *Kalmia latifolia*, the mountain laurel; (2) the genera *Leptobyrsa* and *Stephanitis* are represented in Brazil and in Central America (8); (3) the earliest dated record (1877) is an American one (21); (4) that the first rhododendrons imported into Holland came from America (see below).

Of the European countries, Holland was the first to record, if not to receive, *Leptobyrsa rhododendri* Horv., for, although the 1901 record of the insect at Kingston-on-Thames, England, is earlier than any published Dutch record, it was stated by Raffill (40) that the infested plants at Kingston had been imported from Holland. Schoevers also writes (*in lit.*) that the bug was known fifteen years before Ritzema Bos knew of its presence in Holland, and that it came to that country from U.S.A. on rhododendron varieties, "Flushing", "General Grant" and "James Bateman".

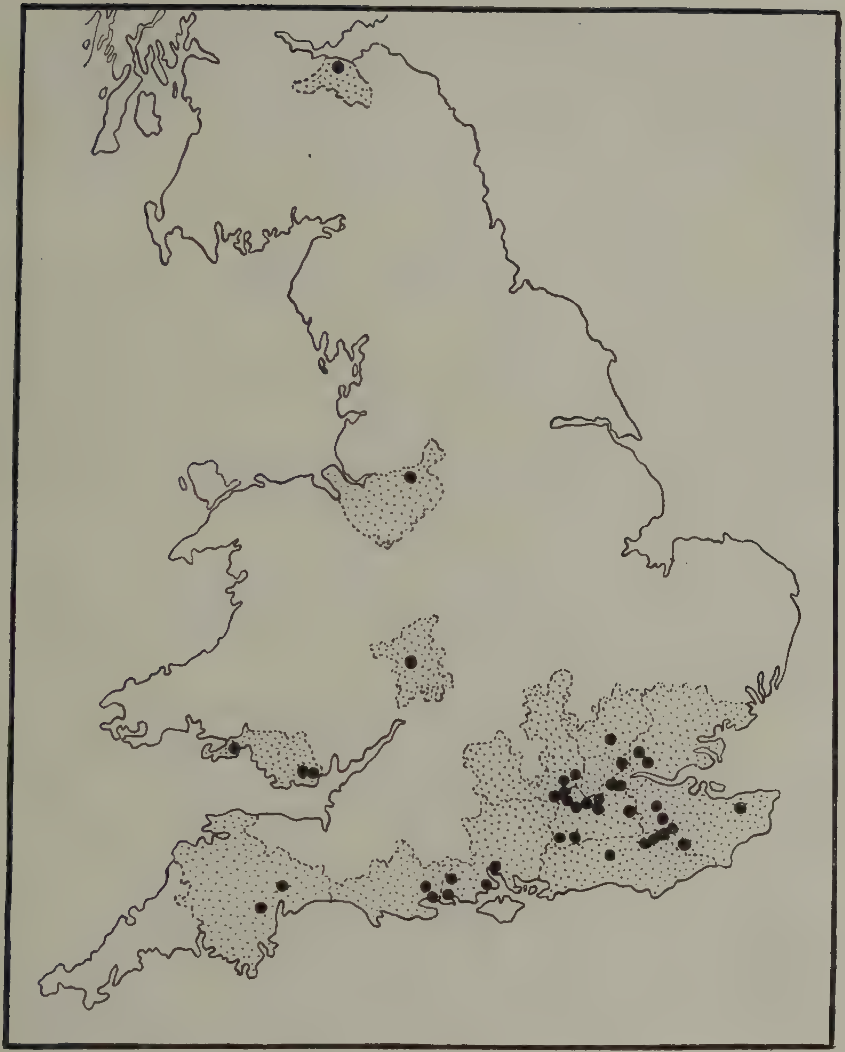
The first French records (16, 33) also stated that the infested bushes came from Holland, while the insect was probably introduced into Germany in the same manner (49) and from the same source.

The factors which caused the insect to become a pest—whether they were the moving of wild rhododendrons to centres of cultivation or changes in the habits of the insect itself—are not known.

III. DISTRIBUTION IN GREAT BRITAIN

Neither *Stephanitis oberti* Kol. nor *S. pyrioides* Scott have been recorded in Great Britain, but *Leptobyrsa rhododendri* Horv. is known to occur in many localities, more especially south of the Thames.

The physical factors which may limit the distribution of the bug in Great Britain are probably mainly those which limit the distribution of susceptible varieties and species of *Rhododendron* (3). In the map (Text-



Text-fig. 1. Map showing known distribution of *Leptobyrsa rhododendri* Horv. in Britain in 1935.

fig. 1) those counties in which the insect has been recorded are stippled; the actual localities where the insects have been found are represented by dots. A list of these localities is given below:

Edinburgh (Edin.); Styal (Cheshire); nr. Worcester (Worcs); Blackpill; Pentyrch; Creigau; Lisvane (Glam.); Chudleigh; Killerton (Devon); Wimborne Minster; Poole (Dorset); Exbury; Southampton;

Ringwood; Bournemouth (Hants.); Hayward's Heath; Upper Hartfield; Eridge (Sussex); Matfield; Hawkhurst (35); Sevenoaks; Tunbridge Wells; Harbledown; Tonbridge (Kent); Camberley; Kingston-on-Thames (40); Kew (54); Woking (17); Wisley (14); Limpsfield; Hindhead; Chiddingfold; Surbiton; Sunningdale (Surrey); Fulham (12) (London); St Albans (Herts.); Windsor; Ascot (Berks.); Slough (Bucks.); Loughton; Gidea Park, Romford (Essex); Enfield (Middlesex).

IV. COMMON NAMES FOR THE INSECT

The following common names have been used for *Leptobyrsa rhododendri* Horv. by various writers:

Rhododendron lacebug (Americano nomina officinalis (26)); rhododendron lace-bug (9); rhododendron bug (18, 19, 22); lace fly (53); lace-wing fly (1, 45); rhododendron fly (20, 52); Catawbiense fly (?) (36); rhododendron-wanze (31); Le Tigre du Rhododendron (16, 33); Japansche luis (6); Japansche wants (7); Japansche vlieg (58); Spaansche vlieg (58).

V. THE ADULT INSECT

Several descriptions of the adult insect are available and, therefore, it is not proposed to add another. The general appearance is shown on Plate XIII, figs. 1 and 2.

Times of appearance and situations on the bushes

The first adults are usually seen, together with fourth, third, second and, occasionally, even first instars, during the last days in June. All stages are then represented, frequently on a single leaf. Like the immature stages the adult is not an active insect, but unlike them there is no inclination to congregate. Indeed the adult migrates from the leaf on which it hatched to other parts of the plant, and by the end of July adults may be found on leaves of all ages (except extremely young ones), although more commonly on those which contain the recently hatched eggs. As August progresses, however, the leaves of the current year (*i.e.* the most distal leaves—those which opened from the leaf buds in the spring) come to bear most of the adults, and this is possibly due to the fact that the insect favours relatively well-lighted positions. Oviposition takes place chiefly on these leaves. The spreading of the insects over a bush seems to be accomplished entirely by walking.

Migration and flight

Migration from bush to bush inevitably occurs if adjacent bushes touch. It is stated (52) that bushes growing in the shade are less liable to infestation than those growing in well-lighted positions, and personal observations confirm this view. In spite of this fact, however, shaded bushes are frequently badly infested. It is also stated (1,18) that rhododendrons growing in woods may be quite free from the bug although situated only a short distance away from a source of infection. Spreading is said to take place more rapidly in a warm, dry summer than in a cold, wet one (19).

There is no direct evidence that migration from bush to bush takes place by flight. J. C. (27) states that the flight is short and takes place only at night; but the insect may be blown by the wind to other plants (45). No other observer has recorded bugs using their wings except to flutter for a yard or two when knocked from a bush. Consequently it is the general belief that wings are little used for migratory purposes. The present writer has observed the insect to fly spontaneously only in the laboratory. In the middle of October 1933, with insects which had been brought in the same day from the field, a few crawled to the tips of the leaves and began a short whirling flight. Again, in the beginning of July 1934, bugs of both sexes which had been reared under glass, if pushed gently off a leaf, flew with a straight and steady flight towards a window at a speed of about 1 yard in 3 sec. (lab. temp. 72° F.). Both occasions on which flight occurred were sunny afternoons. The writer has never seen rhododendron lacebugs flying out-of-doors, and, although bushes have often been shaken, bugs have been seen only to fall inertly to the ground. It is most likely that the distribution of rhododendron lacebugs has been due more to the transference of plants bearing insects, or their fertile eggs, from place to place than to any other single cause.

Mating, oviposition, broods and disappearance

Mating begins in the middle of July. The latest date on which the writer observed a pair *in copula* was September 30 (1933). It is not known as yet if individual insects pair more than once during the summer and autumn.

Oviposition commences towards the end of July. Eggs are laid in rows or long clusters on the underside of the leaf in the tissue at the side of the midrib. Only in rare instances have eggs been seen at the sides of other veins (generally secondary veins, more rarely near the vein endings); they are not deposited actually in the mesophyll between the veins except very occasionally near to the midrib. The majority of eggs are laid on the most distal clusters of mature leaves, *i.e.* those which opened from the bud in the spring immediately before the eggs hatched, and which mature during the summer. Very occasionally eggs are laid on the leaves of the previous year, more especially where the internode between successive, annual clusters of leaves causes the leaves of the two consecutive years to be very close together.

When about to oviposit, the insect seeks a suitable place on the midrib and the ovipositor is erected perpendicularly to the ventral surface of the abdomen. With a saw-like motion the blades penetrate the leaf with great ease, and very little body pressure seems to be exerted upon the ovipositor. After about 1 min. the ovipositor is withdrawn, the terminal structure which bears the anus is evaginated and a drop of olive green faecal fluid is deposited on to the surface of the cap of the egg, which lies flush with the leaf surface. This drop is smeared over the cap by the evaginated anal protuberance, which is moved by deft circular motions of the tip of the abdomen. The fluid eventually dries to form a brown, encrusting scab overlying the cap; such scabs are often confluent, covering the caps of as many as six eggs. Later, in the winter, this scab frequently falls off, leaving the caps of the eggs exposed.

Sorauer (46) states that oviposition occurs in September and October, but Millais (36) and Harding (20) doubt this. The latest date at which oviposition was observed by the writer, however, was October 1, 1933. Mr G. Fox-Wilson (*in lit.*) has observed oviposition taking place in December (1931) at Wisley, and in mild weather it is probable that eggs are laid almost as long as there are bugs about, since females taken on October 30 (1934, see below) contained a few eggs ready to be laid. Thus there is normally a continual deposition of eggs from the end of July well into the autumn.

In Southampton there is only one brood each year. It is true that immature stages are found in July (even an occasional first instar at the beginning of the month), but these are due to late hatchings of the normal over-wintered eggs. In New Jersey, U.S.A., both one and two broods are thought to occur during the year (10), and the general similarity of the life history of the single brooded insect in New Jersey (cf. also for New York (9)) and in England and on the continent of Europe makes it probable that double broods may also be expected both in this and in other European countries if hatching occurs very early in May and if the summer is hot and dry. There is, indeed, a single British record (52) where hatching is stated to have been observed in October.

There is no evidence that adult insects survive the winter, and it is certain that death normally takes place in the autumn or early winter. Many die in the act of oviposition. In 1934 less than a dozen live bugs (females) were found on October 30, in spite of an extensive search over a badly infested area; no live males were seen. Hitherto no over-wintered individuals have been seen by the writer to reappear in the spring.

VI. THE EGG

The following description of the structure of the egg applies to ripe ovarian eggs, except where sections of the cap are dealt with; these appertain to eggs sectioned *in situ* in leaf tissue.

External appearance (Text-fig. 2A)

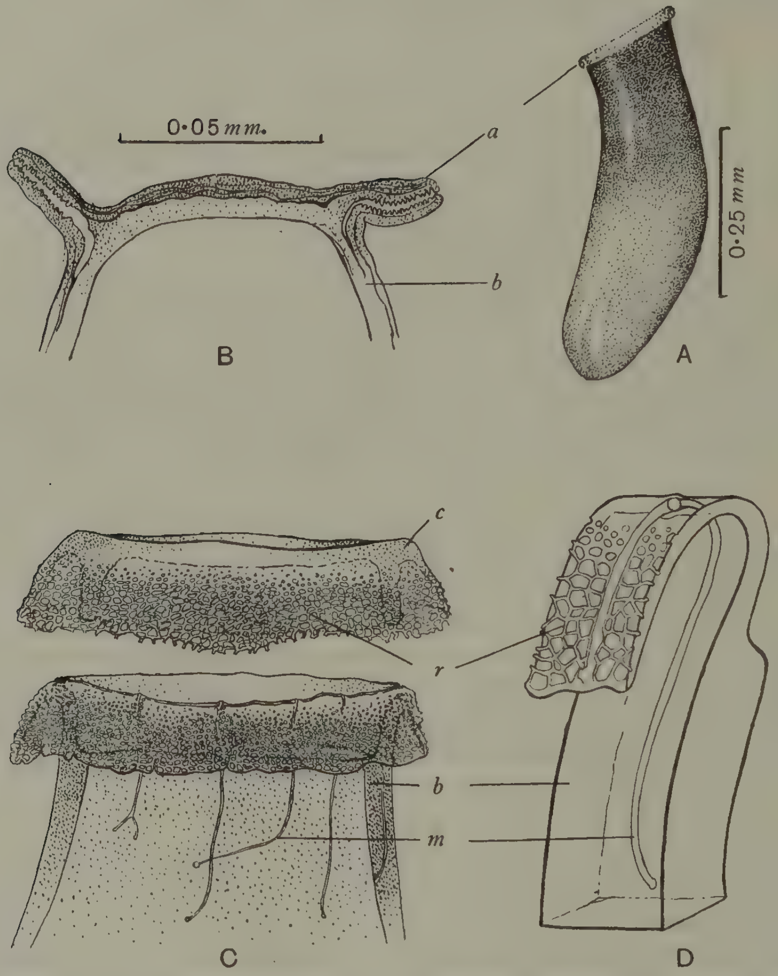
Length 0·5 mm. Lateral width at widest part 0·19 mm. Lateral width of neck beneath cap 0·14 mm. The egg is more or less cylindrical, slightly curved dorso-ventrally. Posterior end bluntly rounded. Neck slightly compressed laterally, bearing a whitish rim anteriorly. In surface view, dorsal region of cap slightly wider than ventral. Peripheral region of a cap a rounded rim, the central region of cap slightly bulbous and not projecting above the level of the rim. Chorion unsculptured, smooth, shiny and cream except for the anterior or neck region which is deep brown.

Microscopic structure

The chorion is uniform in thickness except at the neck where it thickens considerably. Exochorion and endochorion are difficult to distinguish, but it seems that the endochorion is the thicker layer.

The structure of the rim around the anterior edge of the neck where chorion and cap meet is most easily seen when the latter is displaced by pressure upon the cover-slip (Text-fig. 2C). The chorion is seen to be folded back externally, making a narrow collar (about 0·02–0·03 mm. wide), the inner or under surface of which is continuous with the outer surface of the egg. This collar, which is apparently exochorionic, is seen in transparent preparations to possess a lace-like structure of wider mesh towards the free edge. About ten exceedingly fine micropyles (less than 0·002 mm. in diameter) run through the chorion of the neck and open on the inside of the egg at a distance of about 0·07 mm. below the anterior edge of the collar. They occasionally branch once and may cross each other without anastomosing. On reaching the rim the micropyles bend so as to pass into the collar where they become lost in its meshwork.

Sections of eggs (Text-fig. 2B) show that the walls of the meshwork are slightly raised on the outer face of the collar (*i.e.* the face continuous with the inner surface of the egg), and it is possible that the micropyles become shallow, open troughs as they enter the meshwork (Text-fig. 2D), the concave face of the trough being directed outwards. If the micropyles are allowed to fill with air they can be seen as tubes passing from the chorion over the rim and into the collar for the short distance



Text-fig. 2. The egg of *L. rhododendri* Horv. *A*, ovarian egg: note rim anteriorly. *B*, long. section through anterior end of egg: the egg was sectioned *in situ* in leaf tissue hence the rim is not folded back completely, being bent upwards by pressure against the epidermis of the leaf: note raised reticulations internally. *C*, cleared preparation of egg, at anterior end, with cap displaced by pressure of coverslip. *D*, portion of chorionic rim of ovarian egg: rim here completely folded back: note micropyle running through chorion, over rim and among reticulations. *a*, rim. *b*, chorion. *c*, cap. *r*, reticulations. *m*, micropyle.

before the mesh-work begins. Once within the meshes, however, the micropyles cannot with certainty be regarded as tubes.

The cap of the egg consists of a sole-shaped plate with a thick ridge running round it. To the outside of this ridge is a thinner membrane resembling in size and structure the collar attached to the chorion at its anterior end. With the cap in lateral view, the thick ridge is seen to be that portion of the chorion which becomes detached with the cap and which normally fits into the inside of the mouth of the neck. When the cap is in place, the thinner membrane (which is devoid of micropyles) around it fits over the outer surface of the collar on the chorion. Sections indicate that the thinner membrane and the collar of the chorion do not join together at their surfaces, and that even their outer edges are at least not joined continuously.

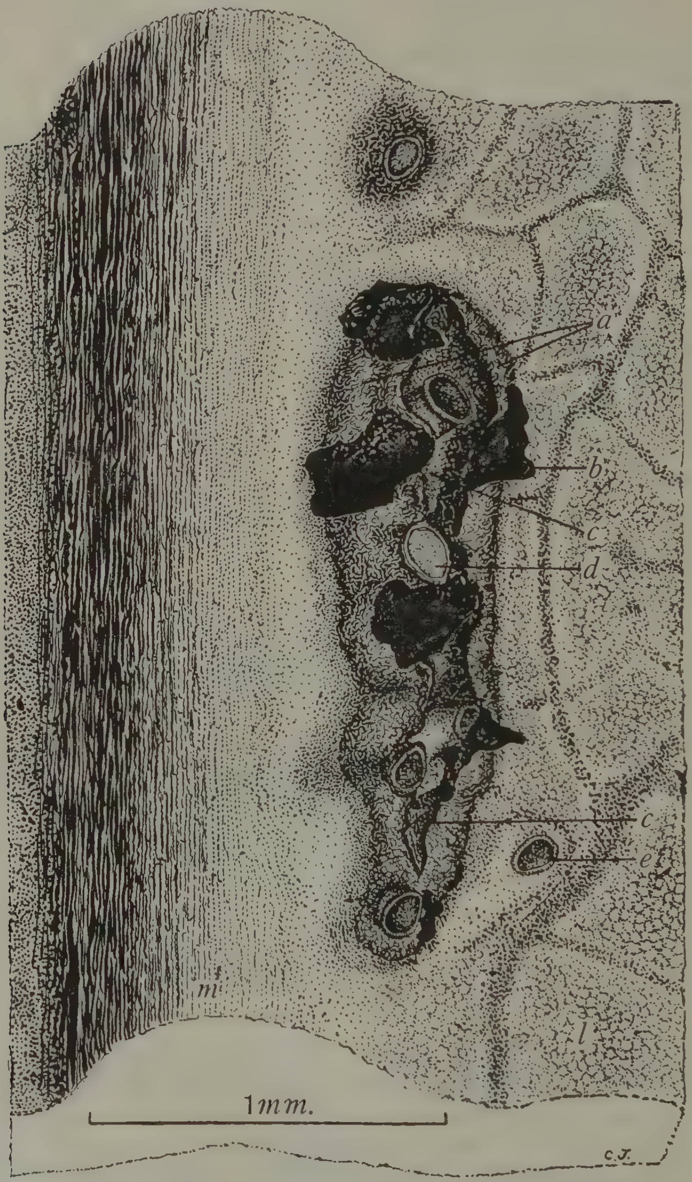
If the egg is allowed to dry and is then mounted in Berlese's fluid it is seen that there are about twenty irregular, hexagonal and pentagonal, dark brown markings around the egg forming a pattern in the chorion, particularly at the neck where the markings are smaller and more distinct than elsewhere.

The egg gall (Text-fig. 3)

It has been recorded by Crosby and Hadley (9) that a corky internal growth surrounds the egg, making no visible alteration to the outside of the leaf. Present investigations have shown that definite gall-like growths are present in certain cases and that considerable external changes are then usually visible.

The mature gall is a raised brown lump along the side of the midrib or, in rare instances, at the side of a minor vein. The brown colour (which is due to the cellular tissue of the leaf, and not to the scabs over the eggs) ends somewhat abruptly before passing into the green colour of the leaf around the gall. Where there is only a single egg the gall is usually quite circular in surface view, but it is more common to find an elongated gall in which are embedded an irregular row of eggs. Galls occur on either side of the midrib, often for the greater proportion of its length, and are of variable size, from less than 1 up to 4–6 mm. long. Sometimes several galls are confluent and each may contain from one to twenty eggs. One typical fairly badly infested leaf contained 154 eggs and bore forty-one galls (sixteen eggs were free from a surrounding gall). The largest gall was 2·5 mm. long and contained thirteen eggs.

Galls frequently have deep cracks running along them, exposing the chorions of the eggs and even the green mesophyll of the leaf. Fungus



Text-fig. 3. Surface view of mature gall along the side of midrib of rhododendron leaf (The Queen). The eggs had over-wintered in the gall and the leaf was of the current year when the eggs were laid. *a*, proliferated gall tissue. *b*, scabs of excrement. *c*, cracks in the gall. *d*, cap of egg. *e*, single egg without surrounding gall: this egg was believed to have over-wintered. *l*, lamina. *m*, midrib.

mycelium is often present in these cracks. Deep holes also occur in the galls, and sections show the dead remains of a much compressed egg in each of them.

The caps of the eggs are normally flush with the surface of the gall. Since at oviposition the caps are level with the ungalled surface of the leaf, the eggs presumably either elongate or are lifted up as the gall tissue proliferates. It is a curious fact, as yet unexplained, that in leaves examined in April, after eggs had over-wintered in their tissue, galls were not formed around every egg. This did not apparently depend solely upon the date at which the eggs were laid, since frequently adjacent to a group of eggs within a well-developed gall one or more eggs, presumably laid at the same time, were devoid of surrounding gall tissue. Moreover, such eggs were not infertile.

Occasionally an egg may be only partially inserted into the leaf, the anterior half projecting above the level of the epidermis. More rarely an egg may be laid horizontally on the leaf surface, without being embedded in the leaf tissue at all.

An examination of the internal anatomy of the gall shows that considerable changes occur in the leaf tissue around the egg (Plate XV). Briefly stated, there is a proliferating zone of leaf cells around the egg resulting in a compact mass of leaf tissue in which the egg is held. Much distortion and compression of the proliferated cells occurs, and their walls and cell contents undergo marked changes. Photographs of sections of eggs *in situ* are given, showing eggs before a gall has formed around them and eggs embedded in a well-developed gall.[1]

A somewhat similar gall, produced by *Heliothrips haemorrhoidalis* Bché on citrus, has been noticed by Rivnay (41).

VII. HATCHING

All the eggs of a batch hatch within an hour or two of each other, and the complete process, commencing with the lifting of the cap, takes about 15 min. The cap is lifted completely from the chorion by a swelling of the embryonic head which appears as a white, shining globule with eyes, antennal bases, labrum and stylet tips visible. Swelling continues in the median line, dorsal to the eyes, and by producing an exceedingly great and curious distortion of the head pushes the cap clear. Pulsations within the swollen head occur until the insect has almost emerged. Then as the embryo bends dorso-ventrally, backwards and forwards, the head begins to collapse. The fluid between the embryo and embryonic cuticle is swallowed, and two spots on either side of the head above the antennal bases are repeatedly pulled into depressions and then released. These curious cephalic movements which are accompanied by a pumping action within the head and by antiperistalsis in the abdomen, may aid in splitting the embryonic cuticle, which now ruptures longitudinally over the thorax. Air bubbles pass rapidly along the pharynx, although pumping movements in the head seem to have stopped. The prothoracic legs and antennae free themselves and the insect climbs on to the leaf. Air still passes down the pharynx as the insect walks away, and imparts a whitish appearance to the thorax and abdomen and causes the latter to be elongated and cylindrical. After about half an hour air is absent from the abdomen which is then excessively small and shrivelled; how this air disappears has not been ascertained. In insects carefully mounted in Berlese's fluid there appears to be no air in the gut of any of the instars when feeding has been in progress for a few hours after ecdysis or hatching. After an hour or two the abdomen becomes more swollen and greenish as plant juices are swallowed, so that by the time the first instar larva is ready to moult into the second larval stage it is twice as long and the abdomen twice as broad as when the insect was small, and shrivelled about half an hour after hatching from the egg. The head width remains the same. At this stage the tip of the rostrum reaches to the very end of the abdomen, and some difficulty is thus experienced by the insect when it attempts to puncture the leaf for the first time.

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[1] Sections of galls were cut from paraffin blocks in preference to celloidin, but lengthy infiltration with wax is necessary. It was found that thick sections (20–28 μ) were more suitable than thin ones.

The chorion of the egg remains within the leaf, and the embryonic membrane with the cap attached to it hangs out of the open neck. Antennal sacs can be distinguished on this membrane.

In Great Britain hatching commences in May (May 17, 1933, May 20, 1934, in Southampton) and continues into June or even into the beginning of July. In Southampton the eggs on the sunny sides of the bushes hatched many days before those on the shady sides, but by June 30 (1933) almost all hatching was over, only very few young first instars being found after that date. The majority of eggs hatched during the first fortnight in June.

VIII. THE IMMATURE STAGES

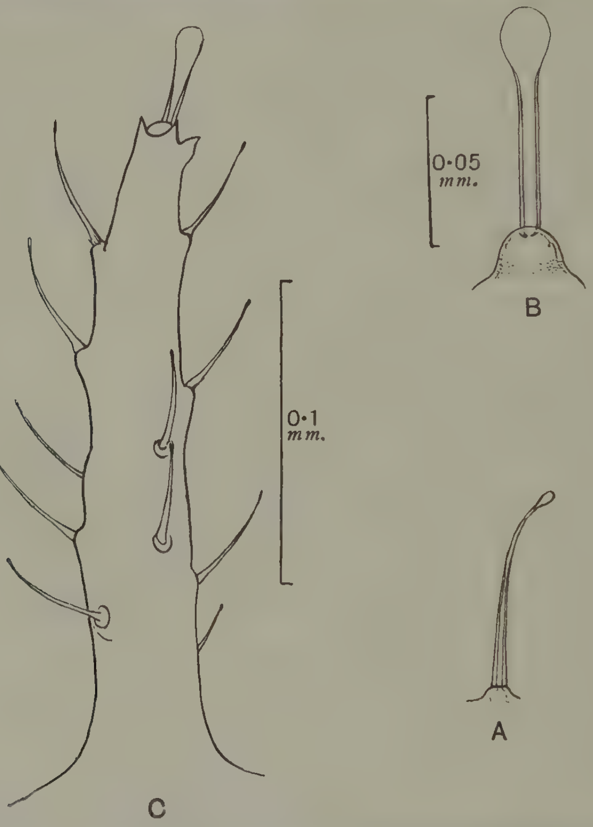
General habits

Feeding does not take place immediately after hatching, but the insects remain clustered at the midrib for about an hour. Then they all migrate to a suitable spot on the underside of the lamina and remain crowded together as close to each other as possible while they feed. Most of the insects which hatch at the same time form such a cluster. The number of insects in a cluster varies from about ten to about fifty for the first three larval stages, and from about five to fifteen for the last stage, when the insects even in the same cluster do not show so strong a tendency to keep close to each other as is the case with the first three stages. These groups of immature stages feed and move about together, leaving a patch of injured cells and a patch of excrement at each of the previous feeding places. It seems that after feeding for a time they move together to a fresh place, which may be a centimetre or two away, possibly because of discomfort due to accumulation of excrement and presumably to exhaustion of the leaf cells providing food.

If individuals of the larval stages are placed on a leaf in as scattered a manner as possible, they become gathered together again after an hour or two in one or more groups. The tendency is most marked in the first and second instars, less so in the third and fourth. This gregarious nature of the larval stages results in the insects usually completing their growth to the adult stage upon the leaf on which they hatched. Members of the fourth instar, however, sometimes migrate to other leaves.

First instar: just before ecdysis (Plate XIV, fig. 1)

Length 0·88–0·9 mm. Translucent pale green or colourless; surface impunctate, shiny. **Head:** Subglobular, pale translucent yellowish or olive green; 0·18 mm. between eyes, slightly more than two-thirds width of thorax. Tubercles five—dark, short and conical; two dorsal, two more closely set near base of brownish clypeus with one median tubercle between them; median with two or three, other tubercles with one, short, club-shaped, apical hair; dorsal tubercles with an additional slender glandular hair between base and apex. On each side of anterior tubercles a longitudinal pale line; parallel until opposite eyes, then meeting between posterior tubercles; thence connected to posterior margin by a single line; a transverse pale line, from each eye to the longitudinal lines where these commence to converge. Eyes fairly prominent, with five separated, red elements. Clypeus visible from above, brown, with a few longish more or less clubbed hairs, each often bearing a terminal fluid globule. On each side of head at posterior margin a brownish latero-dorsal patch. Antennal bases brownish. *Antennae*: 0·57 mm. long, four-segmented; pale yellowish, about two-thirds the length of body; segments I and II very short, of almost equal length; segment III almost twice as long as IV. All but first with long, simple hairs each with a distinct, terminal, fluid globule. Segment IV with additional long hairs (about six of them with terminal knobs), two small blunt spines and a few short hairs distally. Distal half of fourth, distal end of third and whole outer face of second segment smoky brown. **Thorax:** Translucent, almost colourless, yet somewhat glaucous, with smoky latero-dorsal patches (nearer together on meso- and metathorax). On each segment just above coxae, dark, lateral patches, very distinct at dorsal edge; prominent tubercles absent; in early stages of this instar thorax may be as wide as head. Pro- longer than mesothorax. Anterior half of prothorax pale. Usually one (occasionally two) lateral, short, clubbed hair on a minute tubercle on each side of each segment, those on mesothorax more dorsal. Pro- and mesothorax with a similar hair on each side mid-dorsal line. *Spiracles* on posterior margin of pro- and mesothorax; absent from metathorax. **Abdomen:** Subcylindrical, bluntly tapering at anal extremity; pale yellowish or olive green; connexivum colourless or very pale yellow; ten segments (tenth very narrow) in addition to small terminal anus-bearing structure. Later, at



Text-fig. 4. *L. rhododendri* Horv. A, glandular hair from third antennal segment of first instar. *B*, lateral tubercule, bearing clubbed hair, from sixth abdominal segment of first instar. *C*, lateral tubercule from sixth abdominal segment of fourth instar.

widest part slightly wider than thorax. Segment I of same colour as lighter parts of thorax; clubbed hairs absent. Segments II–VIII with a small lateral tubercle on each side (those on eighth the smallest) bearing a single, colourless, clubbed hair, longer than those on head tubercles. Segments III and IV with smoky brown, laterally incised centre patches. Segments II, V, VI, and VIII each with a dorsal median tubercle bearing two clubbed hairs; median tubercles on fifth and sixth with confluent, brown, basal patches. Segment IX with two latero-dorsal tubercles each with a clubbed hair; laterally, on each side of segment a minute tubercle with a small clubbed hair. Segment X with four longish clubbed hairs and one or two ventral, glandular hairs on posterior margin. *Spiracles* on segments II–VIII. Transverse, median, abdominal glands between segments III and IV, and IV and V; anterior gland the larger. A few sessile shiny globules on tergites. Underside of abdomen with a few simple hairs. *Legs*: Subequal. Yellowish. Coxae smoky brown. Tips of tarsi brownish. Tibiae as long as femora, both with a few short, simple hairs distally. Tibiae each with two short, outwardly directed, clubbed hairs proximally. Tarsi two-jointed with a few simple and three or four glandular hairs. *Rostrum*: 0·51 mm. long from base of labrum.

Second instar: about half-way between successive ecdyses
(Plate XIV, fig. 2)

Length 0·9–1·07 mm. Shiny, translucent, very pale greenish yellow or colourless on lighter parts; dark brown elsewhere. **Head**: Subglobular, 0·23 mm. between eyes, almost two-thirds width of thorax. Shiny, yellow or smoky pale olive green; dusky brown marking on each side near posterior margin. Five long, dark brown, cylindrical tubercles, the anterior pairs projecting forwards; posterior pair longest (about 0·1 mm.) each with about a dozen glandular hairs from base to apex (each hair with a terminal fluid globule) and a short clubbed or trumpet-shaped apical hair. Two anterior tubercles shorter, each with a few simple glandular hairs and one or two apical, clubbed hairs. Median tubercle, near anterior pair, of intermediate length with a few simple globule-bearing hairs from base to apex and two short, clubbed, or trumpet-shaped apical hairs. Eyes prominent, with at least five separated red elements. Clypeus brown, just visible from above, with a few long, clubbed hairs each with a fluid globule; labrum brown, with a few simple hairs. Pale, yellowish lines present, of similar pattern to those on head of first instar. Suggestion of a shallow longitudinal groove between posterior tubercles. *Antennae*: 0·76 mm. long; slightly more than two-thirds the length of body; resembling those of first instar, slightly more hairy, long terminal hairs on segment IV less obviously clubbed. **Thorax**: Surface with extremely minute raised dots. *Prothorax* translucent, colourless to very pale yellow, slightly glaucous; transverse, produced laterally into a slightly uptilted tubercle, each with an apical clubbed hair and one or two simple glandular hairs below. Usually a clubbed hair on each side of the mid-dorsal line. Anterior margin entire, posterior margin convex, minutely indentate and slightly incised at centre. *Mesothorax* transverse; lateral tubercles as on prothorax, but slightly larger, uptilted and with dark brown or black bases occupying width of segment; each tubercle with a short, terminal, trumpet-shaped hair and two simple glandular hairs below. A prominent dark tubercle on each side of mid-dorsal line, each with a terminal clubbed hair and a few longer glandular hairs each with a terminal, fluid globule, from base to apex. *Metathorax* slightly shorter than mesothorax, brownish, with pale central area and a pale, translucent, yellowish connexivum; sometimes with a short, clubbed hair near posterior angle on each side; posterior margin very slightly biconvex. *Spiracles*, near posterior margin of pro- and mesothorax; absent from metathorax. **Abdomen**: Above, more or less triangular, later becoming ovate. Segments, except first, smooth and shiny, with a few sessile globules; much more convex ventrally than dorsally. Connexivum translucent, pale yellowish. Segment I yellowish, short, resembling metathorax, but paler, and without hairs, tubercles or brown patches; seen laterally the tergum is very strongly convex, the surface with minute raised dots. Segment II longer than I, pale whitish yellow, each side with a single minute tubercle bearing a clubbed hair; a single, dark, prominent median tubercle (with a basal, transverse brown marking) often with one or two simple glandular hairs, each with a terminal fluid globule and also one or two short, apical, clubbed hairs. Segments III–X dark yellowish-brown (III–VI very dark) except for colourless to pale translucent olive green connexivum. Segment III short, no prominent tubercles, but on each side, a minute tubercle with a clubbed hair. Pale transverse, median, abdominal glands as on first instar. Segment IV with a lateral tubercle on each side about as long as lateral mesothoracic tubercle and with an apical clubbed hair and usually two simple hairs nearer the base. Segment V similar to IV but with a long cylindrical, median tubercle leaning slightly forwards with a few simple hairs with terminal fluid globules and two apical, short clubbed hairs. Segment VI similar, median tubercle somewhat shorter than that on segment V and with fewer hairs. Segment VII paler, with similar lateral tubercles; median tubercle absent. Median tubercles on fifth, sixth and eighth about twice the size of the lateral tubercles on the same segments. Segment VIII with typical lateral tubercles, and median tubercle, with several simple glandular hairs and two short apical clubbed hairs. Segment IX with two smaller latero-dorsal tubercles each with two or three fairly long clubbed hairs, and a simple glandular hair. Segment X very narrow, short, no tubercles, but with four fairly long clubbed hairs. Underside of abdomen smooth with a few hairs. *Spiracles* on segments II–VIII. *Legs*: Similar to those on first instar; slightly more hairy. Coxae dark brown. *Rostrum*: 0·61 mm. long from base of labrum.

Third instar: about half-way between successive ecdyses
(Plate XIV, fig. 3)

Length, excluding head tubercles, 1·2–1·6 mm. Dark brown, yellowish or colourless lighter parts. Shiny. **Head**: Above subglobular, yellowish olive brown; 0·29 mm. between the eyes; slightly more than half the width of prothorax. Five long, cylindrical dark brown or black pointed tubercles, the posterior and median pointing slightly forwards, the anterior pair projecting directly forwards. Posterior and median tubercles about the same length, anterior slightly shorter; all tubercles from base to apex with numerous glandular hairs (more scanty on anterior pair) each with a terminal fluid globule; one apical, short, stout clubbed hair on each tubercle except the median, which bears two such hairs. Eyes rather prominent, rounded, each with numerous separated red elements, yellowish between. Clypeus scarcely visible from above, dark brown, with a few long, clubbed hairs. Pale yellowish lines (sometimes scarcely visible) similar to those on previous instars, but lines on each side of more anterior tubercles diverge strongly as they pass forwards from their union opposite the eyes. A brownish patch at posterior margin of head on each side. Shallow longitudinal groove between posterior tubercles scarcely visible. *Antennae*: 1·3 mm. long; as for second instar but segment I shorter than II. Long terminal hairs on segment IV apparently not clubbed as in first instar. **Thorax**: Prothorax transverse, approximately 0·22 mm. long and 0·54 mm. across from tubercle bases. Translucent pale yellow, centre colourless; with minute raised dots. A prominent lateral brownish, uptilted tubercle directed slightly forwards, on each side near posterior margin, bearing several slender glandular hairs and a short apical clubbed hair. At the centre, slightly posteriorly, on each side of mid-dorsal line, a short clubbed hair on a minute tubercle; one or both hairs may be quite absent. Surface of prothorax with small sessile, round, shining globules. Lateral margins slightly convex; posterior margin with a slight convexity at the centre. Margins smooth, but for the minute raised dots over the whole surface except a narrow longitudinal region down the centre and a narrow transverse region across the middle of the prothorax. *Mesothorax*: Width, from lateral margin of anterior wing rudiments, wider than prothorax. The small central area, translucent pale yellow or colourless, about half the length of the prothorax; minute raised dots over dorsal surface except on wing pads. A long, black tubercle on each side of the median line with numerous glandular hairs each with a terminal fluid globule, and a short apical, clubbed hair. Anterior wing rudiments almost circular, smooth, blackish-brown, reaching to the first and later to the second abdominal segment; outer, strongly convex, lateral margins entire; at middle point on each side, a long, single, lateral, uptilted tubercle, bearing several slender glandular hairs, and an apical, short, clubbed hair. One or two simple glandular hairs laterally on the outer margin of anterior wing-pads, anterior to the lateral tubercles. Sessile shining globules on surface of wing-pads. *Spiracles* on pro- and mesothorax, towards posterior margin. *Metathorax*: Shorter than central area of mesothorax; seen laterally, tergum strongly convex; translucent pale yellow, brownish under anterior wing rudiments. Minute raised dots on posterior half of segment, reaching towards anterior margin at centre. Posterior margin very slightly concave, scarcely incised at centre. Posterior wing rudiments brownish, completely beneath anterior pads except near base; a clubbed hair on posterior corner on a minute rounded tubercle. A few, sessile, shining globules on tergum. **Abdomen**: Ovate, especially later. Brownish black, shiny; dingy yellow or light brown on lighter parts. Connexivum yellowish brown. Segment I short, resembling metathorax; tergum strongly convex, seen laterally. Posterior centre region colourless. Tubercles and glandular hairs absent; sessile globules occasionally present. Segment II with a minute, lateral, rounded tubercle bearing a short, stout clubbed hair, on connexivum on each side near posterior margin; a long blackish-brown median tubercle near posterior margin, with two apical, short clubbed hairs and a few slender glandular hairs below; tergum brown around base of tubercle, yellowish elsewhere. Segment III like II but median tubercle absent; brownish but for connexivum. Segments IV–VIII with a dark brown sub-cylindrical slightly uptilted, lateral tubercle near posterior margin on each side; these tubercles which bear slender glandular hairs each with a terminal fluid globule, and a short apical clubbed hair, are not as long as prothoracic lateral tubercles. Median tubercles on segments II, V, VI, VIII, that on second segment smallest and with fewer glandular hairs; short apical clubbed hairs on median tubercles as follows: on segment V, one; segment VI, two; segment VIII, two, three or four: these clubbed hairs are somewhat longer than those on lateral tubercles and the median tubercles themselves are twice as large as lateral tubercles on same instar. The three more posterior median tubercles are the same size, and nearly twice as large as those on second instar. Segment IX with two latero-dorsal tubercles each often with three longish clubbed hairs. Segment X small, with four longish slender, clubbed hairs; tubercles absent. Abdominal glands as on previous instars. *Spiracles* on segments II–VIII. Underside of abdomen smooth, with longish slender, glandular hairs towards posterior margins of segments. *Legs*: As for first and second instars, and in same proportion to body length; tibiae slightly more hairy. *Rostrum*: 0·68 mm. long from base of labrum.

Fourth instar: about half-way between successive ecdyses

(Plate XIII, fig. 3)

Length excluding head tubercles, 2·1 mm. Dark brown or black, yellowish or light brown lighter parts. Scattered over the head, pronotum, wing pads and abdomen are small sessile, glistening globules. **Head**: Subglobular, 0·32 mm. between eyes. Brown on darker parts; yellowish pattern as on head of third instar. Antennal prominences distinct. Five very long (about 0·2 mm.), cylindrical, slender, very dark brown or black tubercles each with a short apical clubbed hair and with glandular hairs from base to apex each with a terminal fluid globule; posterior and median longest; median and anterior clustered together, projecting forwards. Eyes subglobular with numerous dull red elements. A brown patch on each side of head at posterior margin. Clypeus visible from above, dark brown with a few, fairly long, clubbed hairs. *Antennae* yellowish, approximately 2 mm. long. Segments I and II with two or three glandular hairs distally, each with a terminal fluid globule; brown on outer faces. Segment II about half as long as I. Segment III more than twice as long as IV and with numerous glandular hairs, each with a terminal fluid globule; distal end dark brown and sometimes a dark band half-way along segment. Segment IV wider and pointed at dark brown distal half; in addition to glandular hairs a few very long apparently unclubbed hairs near apex and a few apparently non-glandular spines at extreme tip. **Thorax**: *Pronotum* 0·91 mm. broad. Well developed, transverse, broadly shield-shaped or pentagonal; slightly wider posteriorly. Yellowish, more or less transparent with a pale central line. Anterior margin sinuate. Lateral margins slightly convex and somewhat elevated; a long dark brown cylindrical, more or less upright tubercle on each lateral margin near latero-posterior angles; tubercles often of unequal sizes and with slender hairs each with a terminal fluid globule and a short apical clubbed hair. Antero-lateral margins often with three or four simple glandular hairs. Posterior margins slightly raised, strongly produced at centre, there reaching posterior margin of anterior wing rudiments. A semicircular convexity about as wide as the head and strongly arched to a median ridge, at centre of pronotum anteriorly; a much narrower raised median region with apex at centre of pronotum, posteriorly. On each side near latero-anterior angles, a distinct depression. Dorsal surface covered with minute raised dots and sessile globules. Often on each side of mid-dorsal line opposite lateral tubercles a minute tubercle with a short clubbed hair. *Mesothorax* transparent; wing rudiments reaching to fifth abdominal segment, outer lateral margins entire, slightly convex with a number of glandular hairs anteriorly; a long dark, more or less upright tubercle of variable length, with glandular hairs and an apical, clubbed hair, on each lateral margin in a slightly posterior position. A tubercle on each side of mid-dorsal line just behind posterior margin of pronotum and bent slightly forwards each with numerous glandular hairs and an apical clubbed hair. Wing rudiments transparent yellowish brown, dark at tips and bases. A minute raised cone towards the centre of each wing pad opposite first abdominal segment. *Spiracles* on posterior margins or in intersegmental sutures of pro- and mesothorax. *Metathorax*: Short, pale yellowish, only a small section showing between anterior wing rudiments; the latter also completely cover posterior wing rudiments. Posterior margin colourless with shallow median incision. **Abdomen**: Triangular, pointed towards anal extremity; connexivum translucent, broad, yellowish, with brown posterior borders on each segment. Central area dark brown or black. Segment I short, with a median, transverse colourless swelling, strongly convex when seen laterally; like metathorax in surface characters.

On each side of segments II and III a lateral clubbed hair on a minute tubercle. A long, lateral, cylindrical tubercle with slender glandular hairs and a terminal, short, clubbed hair on each posterior angle of segments IV to VIII; these tubercles are smaller than either median abdominal or head tubercles. Segment IX with a pair of latero-dorsal tubercles often with more than one apical clubbed hair. Segment X very small and short with four longish, slender clubbed hairs. Segments II, V, VI and VIII each with a median dorsal tubercle, that on II shortest and with only two or three glandular hairs, and one or two apical, clubbed hairs. Abdominal glands as for previous instars. *Spiracles* on segments II–VIII. On each tergum except the first, a group of sessile globules on each side of mid-dorsal line. Underside of abdomen slightly more hairy than in preceding instars. *Legs*: Sub-equal, yellowish. Slightly more hairy (with apparently glandular hairs) than in preceding instars. Tibiae as long as or slightly longer than femora; tips of tarsi brown. Coxae brownish. *Rostrum*: 0·81 mm. long, from base of labrum.

Remarks on the instars

The smaller details in the above descriptions, *e.g.* shape, position and number of hairs, can only be observed safely under relatively high magnifications (× 430). For such details mounts made in Berlese's fluid were used.

The fluid globules present on many hairs, particularly those without obviously clubbed ends, are of a viscid nature, and increase and decrease in size during the life of a larva. Immature insects, therefore, are often encumbered with lumps of dried excrement or cast larval cuticles which become stuck to these globules as the insects wander over the leaf surface. In the third and fourth instars there is some variation in the shape of hairs which were definitely clubbed in the two previous instars, the tendency being for such hairs to become somewhat shorter and more trumpet-shaped (Text-fig. 4C).

A few incorrect statements concerning the immature stages, which exist in the literature on *Leptobyrsa rhododendri* Horv., may be mentioned.

Crosby and Hadley(9) stated that lateral tubercles exist on all the abdominal segments except the first. There is, however, a very small and short tenth segment (best seen in cleared, unmounted, cast larval skins) which bears no prominent tubercle but has four long clubbed hairs upon it. This segment was apparently missed by the above writers. Heidemann(21), in a very brief description of the second instar, wrote that all abdominal segments possess lateral tubercles; in this case, the first and tenth segments, which are free from tubercles were apparently missed. The same writer was then led to suppose that the median dorsal tubercles were borne on the first, third, fourth and sixth abdominal segments instead of on the second, fifth, sixth and eighth. Heidemann also stated that pores exist on the tubercles, but by comparison of his figures with preparations of larvae it is apparent that he mistook the pale round seta-bases for pores.

Concerning the fourth instar, both Heidemann and Theobald(55) made the same error as Heidemann did in the case of the position of the median tubercles of the second instar. Theobald, moreover, stated that there are tubercles at the apex of each wing pad, and that there are four pairs of lateral and a pair of terminal tubercles on the abdomen; he obviously thought that the first pair of lateral abdominal tubercles were situated upon the wing pad. Heidemann's remark that the wing pads of the fourth instar reach to the third segment is also incorrect, since even in instars which have immediately moulted from the third larval stage, the wing pads (anterior) clearly reach to the fifth abdominal segment.

Neither parasites nor predators of immature or adult stages of *Leptobyrsa rhododendri* Horv. have been found, nor does the literature indicate that any are known. Species of *Stephanitis*, however, are preyed upon by *Stethoconus* spp. (Hemiptera), and Schumacher(43) has suggested that *Stethoconus cyrtopeltis* Flor. preyed upon *Stephanitis oberti* Kol. in Livland.

Moulting periods

The following periods apply to insects kept in the greenhouse during the latter half of May and the first half of June.

First instar (48 insects)	6–8 days (a few 5 days).
Second instar (45 insects)	4–6 days.
Third instar (11 insects)	3–6 days.
Fourth instar (9 insects)	9–10 days.

IX. SUMMARY

1. A brief review of the literature dealing with the history of *Leptobyrsa rhododendri* Horvath as a pest is given. The original home and subsequent dispersal are discussed.

2. The distribution of the insect in Great Britain, as known to the author, is given.

3. The bionomics of the insect are dealt with.

4. Detailed descriptions of the structure of the egg and its position in the leaf tissue are made, together with a short description of the egg gall and an account of the hatching process.

5. The external appearance of each of the four instars is described in detail. Comments upon the descriptions of the immature stages of previous workers are given.

6. The list of references contains all the more important papers which deal with the insect mainly from general economic and bionomic aspects.

X. ACKNOWLEDGMENTS

I wish to express my gratitude to Prof. S. Mangham for the many ways in which he has given help, to Mr G. Fox-Wilson for numerous kindnesses and at whose suggestion the work was undertaken, and to Mr A. E. Clarence Smith for the photographs on Plate XV. To Mr W. E. China I am grateful for help with the synonymy of the insect.

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EXPLANATION OF PLATES XIII–XV

PLATE XIII

Fig. 1. Lateral view of head and thorax of adult male *Leptobyrsa rhododendri* Horv.

Fig. 2. Adult female, *L. rhododendri* Horv.

Fig. 3. Fourth instar, *L. rhododendri* Horv. (5th day).

PLATE XIV

Fig. 1. First instar *L. rhododendri* Horv. (6th day).

Fig. 2. Second instar *L. rhododendri* Horv. (3rd day).

Fig. 3. Third instar *L. rhododendri* Horv. (3rd day).

PLATE XV

Fig. 1. Transverse section leaf of rhododendron (The Queen), with eggs *in situ*, before gall cells had commenced to proliferate, *i.e.* egg freshly laid. Stained in light green and safranin.

Fig. 2. Transverse section leaf of rhododendron sp., with eggs *in situ*. The gall tissue had proliferated and had formed a mature gall: the eggs had over-wintered in the leaf. Stained in cyanin and eosin.

Fig. 3. Transverse section leaf of rhododendron (Caractacus) through a mature gall but between eggs. Stained in cyanin and eosin.

a, egg; *b*, gall tissue; *c*, proliferating zone of gall; *d*, palisade tissue of upper side of leaf.

(*Received October* 24, 1935)

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[1] The original reference has not been seen; it is possessed by no library in London or Oxford.

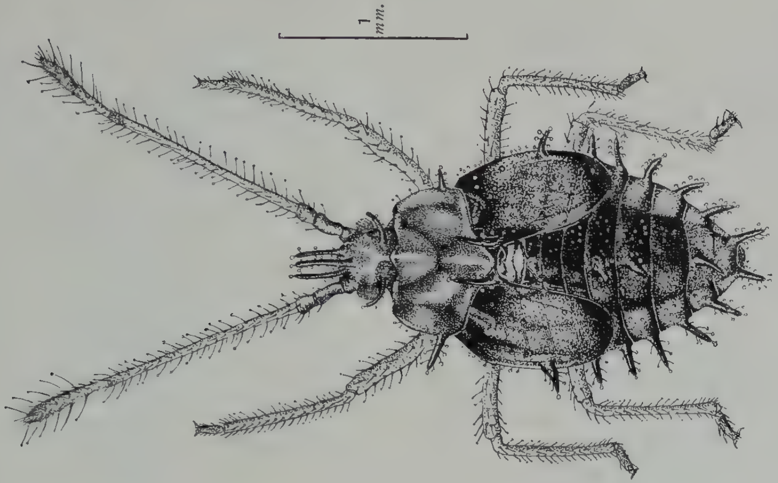
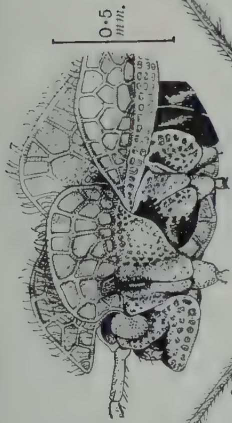


Fig. 3.

Fig. 1.



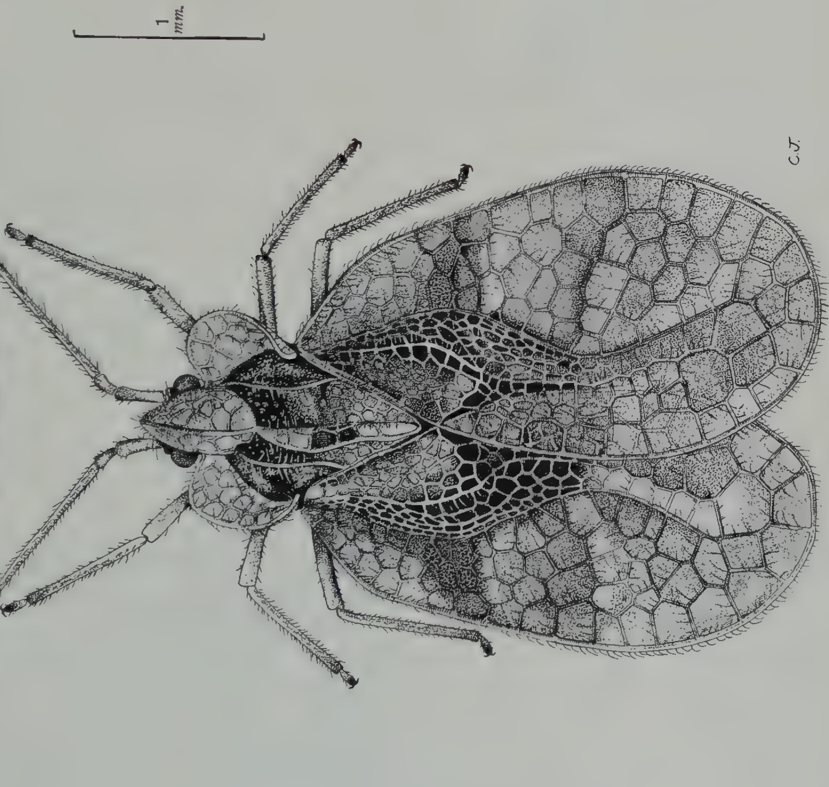


Fig. 2.

JOHNSON.—The Biology of *Leptobyrsa rhododendri* Horvath (Hemiptera, Tingitidae), the Rhododendron Lacebug (pp. 342–368).

PLATE XIV

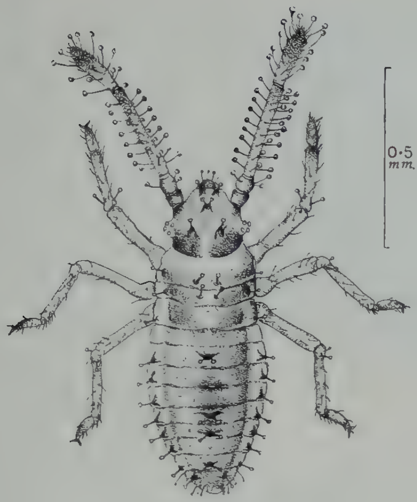


Fig. 1.

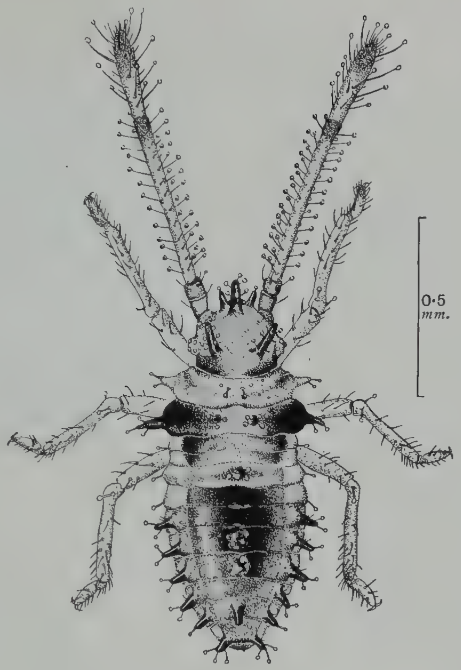


Fig. 2.

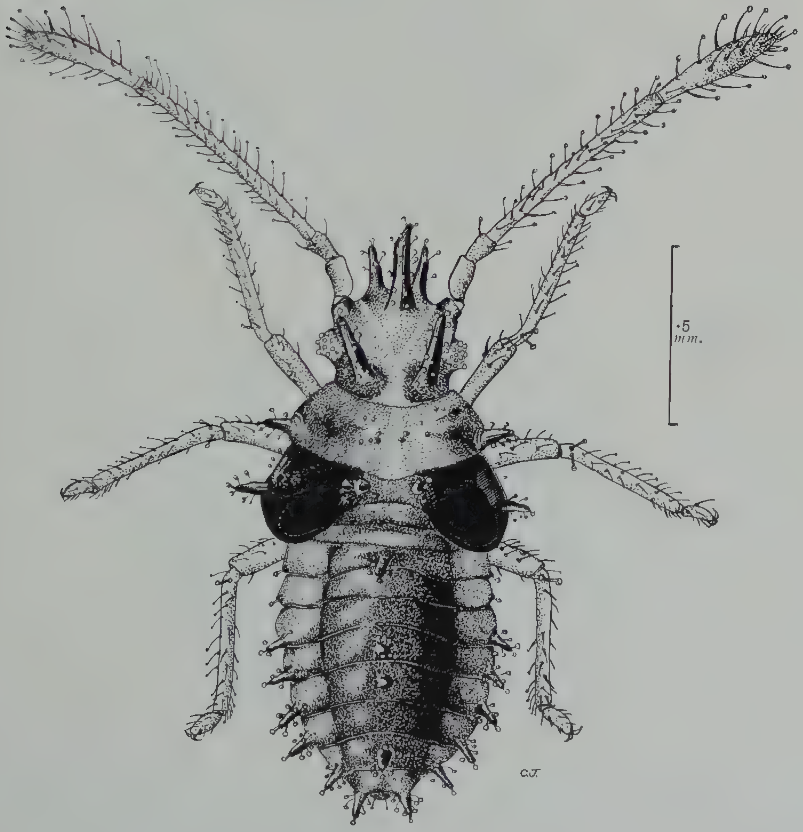


Fig. 3.

JOHNSON.—The Biology of *Leptobyrsa rhododendri* Horvath (Hemiptera, Tingitidae), the Rhododendron Lacebug (pp. 342–368).

A STUDY OF THE FOOD RELATIONS OF THE *LYCTUS* POWDER-POST BEETLES

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(With Plate XVI and 4 Text-figures)

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INTRODUCTORY

COMPARATIVELY little work has been done on the food relations of insects in general, and our knowledge of the subject with regard to wood-boring insects is, as yet, particularly sparse. Several recent researches, however, have thrown considerable light upon the physiology of digestion in wood-borers, and the subject has become one of great interest in view of the unexpected results, particularly in connection with the role of so-called symbiotic micro-organisms. Uvarov (33) in his summary of the literature up to 1927 upon insect nutrition comes to the following conclusions concerning wood-boring insects: "To summarise, it is known that the nutrition of the vast majority (perhaps of all) of wood-feeding insects is intimately connected with their symbiotic micro-organisms, but the physiological side of their nutrition remains entirely unknown. Indeed, all that is known is that the insects, apparently feeding on wood, are not actually using wood directly as food. There is, however, no evidence at all as to what substances they do, in fact, feed on; these may be particles of wood partially digested by micro-organisms, or their secretions, or their excretions, or even the micro-organisms themselves. In other words, wood-eating insects must be classified as insects of absolutely unknown feeding habits. This is an astonishing conclusion at which to arrive with regard to a group of insects of great economic importance and one which is very widely spread and of common occurrence." This general concept of the possible importance of micro-organisms, although still holding good for some families of wood-borers, is rapidly losing favour in the case of others. Furthermore, it has now been shown that some insects are capable of deriving their nourishment directly from wood.

Since the publication of Uvarov's summary, several important papers dealing with digestion in wood-boring insects have appeared. Particular attention has been paid to the larvae of the Cerambycidae, probably because the larger size of many species makes them suitable for this type of investigation. The work of Falck (8), Horn (15), Ripper (27), Müller (25) and Mansour and Mansour-Bek (21) shows that a cellulose is of common occurrence in the larval gut of members of this family: indeed, only one species has so far been recorded (21) as not possessing such a ferment. In the Anobiidae, Campbell (5) has shown and Ripper (27) confirmed that cellulose is broken down in the larval gut of *Xestobium*, and Falck (9) records similar results for *Anobium*. Campbell (5) has also proved by chemical methods that larvae of the family Lyctidae cannot digest the constituents of the cell wall, and Wilson (35), extending the work of Mer (23, 24), has recently shown that starch in wood forms the principal food of *Lyctus* larvae. Mansour and Mansour-Bek (22) have recently reviewed the whole subject of digestion of wood by insects, and it is now clear that the larvae of some wood-borers can break down certain cell wall components and use the resulting products as a source of nourishment, while the larvae of other species are unable to hydrolyse the constituents of the skeletal substance and must rely for food upon cell contents or the breakdown products from wood caused by other agencies such as fungal decay, a subject which has, as yet, been little investigated.

Wood eating involves the organism in adaptation to a highly specialised environment and diet, and for some time past it has been considered by many authors, particularly Buchner (3) and his co-workers, that, with reference to the physiology of digestion, this specialisation is bound up with the presence in many wood-borers of micro-organisms such as yeasts, bacteria and fungi. The relation between host and micro-organism was thought to be essentially symbiotic, the host providing shelter for the micro-organism and the micro-organism helping the host to utilise a refractory diet. The more recent researches of Ripper, Müller and Mansour, however, show that some insects with symbionts cannot digest the cell-wall constituents of wood, and that other insects with no symbionts can hydrolyse certain components. Both Ripper and Müller suggest that the so-called symbionts are more in the nature of hereditable parasites or commensals, but until more information is available the question must remain open, although there now seems little doubt that in some species of wood-feeding insects the micro-organisms cannot be of any value to the host with respect to digestion.

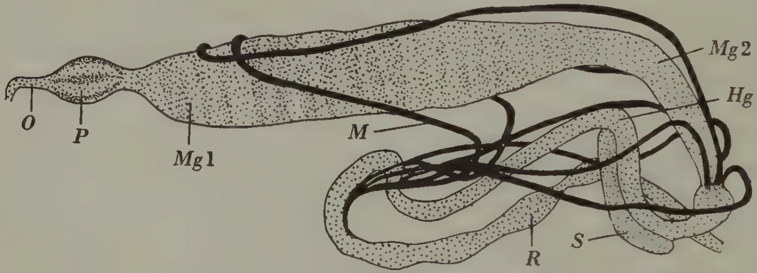
Most of the work so far published deals with Isoptera, Anobiidae and Cerambycidae, but the present paper gives the results of an investigation into the food relations of another family of wood-borers, the Lyctidae.

THE *LYCTUS* POWDER-POST BEETLES

The economic importance of these insects has been discussed by Fisher, Cann and Parkin (11). Recent work on their biology is given in papers by Fisher (10), Kojima (17) and Parkin (26), each of which contains a list of references to the subject. For the purposes of this paper, however, it is desirable to summarise very briefly the pertinent facts of the life history and habits. The beetles emerge from infested wood mainly during June, July and August. They live 3–6 weeks, during which time copulation and oviposition take place. The eggs are laid in the vessels of the sapwood of certain recently seasoned hardwoods, *e.g.* oak, ash, elm, walnut, etc., and hatch in 8–12 days. The larvae tunnel in the direction of the grain, reducing the wood to a fine powder which is packed in the galleries. With very rare exceptions the attack is always confined to the sapwood. The pupal period lasts 3–4 weeks, after which the beetles emerge and recommence the cycle. In Great Britain there is normally one generation per year. Various methods of control are in use, and the present investigation was undertaken to ascertain what substances in wood are utilised as food by *Lyctus* larvae in order to consider ultimately the possibility of treating the wood in some way to remove these substances or render them no longer available in suitable form. If such treatment were practical and economic it would then be possible to render all sapwood immune from *Lyctus* infestation with a considerable saving to the industries concerned.

Material

Lyctus larvae and beetles required for the work were obtained from stocks of infested English and American hardwoods kept at the Forest Products Research Laboratory. The adult beetles were identified with the aid of Kraus and Hopkins' (18) key and by comparison with specimens, the identity of which was determined by Monsieur P. Lesne, Museum National d'Histoire Naturelle, Paris, and Mr W. S. Fisher, U.S. National Museum, Washington, D.C., to whom thanks are due for their willing help. There is at present no means of identifying the larvae of species of *Lyctus*, but the material probably consisted of a mixture of *L. brunneus* Steph., *L. linearis* Goeze, and *L. planicollis* Le C.

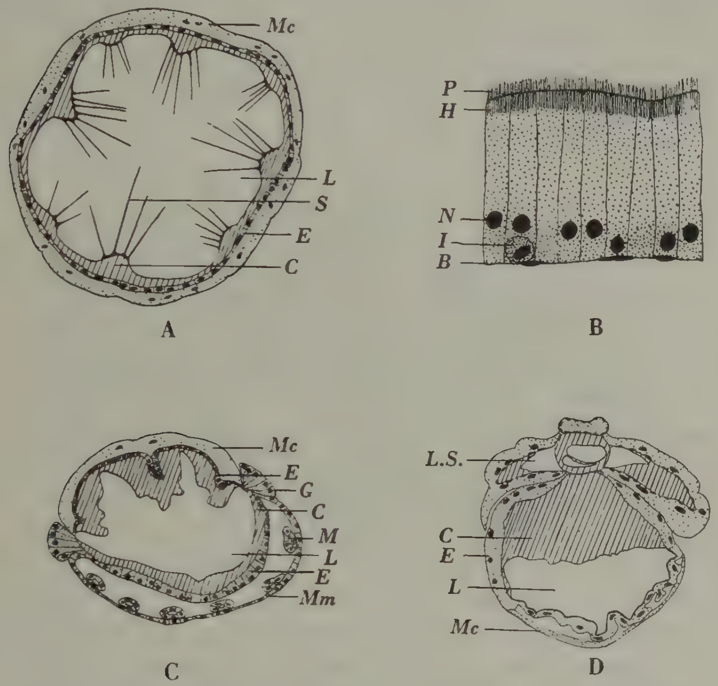


Text-fig. 1. Larval gut of *Lyctus*. *Hg*, hindgut; *M*, Malpighian tube; *Mg* 1, *Mg* 2, anterior and posterior portions of midgut; *O*, oesophagus; *P*, proventriculus; *R*, rectum; *S*, paired sacs applied to hindgut. (× 17.)

The larval digestive system

Apart from a short description by Kojima (17), no work has been done on the morphology and histology of the larval gut. The mouth leads into a short, narrow oesophagus (Text-fig. 1), the thin walls of which are composed of flattened epithelium with a few longitudinal muscles externally and a delicate chitinous intima internally. The oesophagus opens into a small globular proventriculus (Text-fig. 2A), having a thick chitinous lining beset with eight longitudinal bands of backwardly projecting stout setae which probably help to propel the food into the midgut and prevent regurgitation. The wall has a middle layer of flattened epithelial cells and a thick outer coat of circular muscle fibres.

The epithelial wall of the proventriculus projects slightly in the form of a funnel-like valve into the midgut, which is very large and usually full of brown digestive juice containing many small wood particles. The midgut shows two portions, the anterior of which is distended and characterised by the presence of irregular transverse striations, while the posterior is formed of a simple narrower tube; there is no distinct line of demarcation. The wall of the anterior part (Text-fig. 2B) is formed of glandular columnar cells. During the non-secretory phase, there is at the apex of each cell a striated hyaline area from which projects a fringe of numerous, closely set cilia-like processes. In the actively secreting phase the hyaline area disappears, the fringe is much shorter, and the cells are filled with darkly staining basophile granules which rapidly disappear if the larva is starved for a few days. The posterior portion of the midgut is similar to the anterior, but all the cells are shorter, except at the posterior end where they increase somewhat in height. Between the bases of the glandular cells along the whole length of the midgut can be seen scattered small groups of darkly staining interstitial cells which eventually give rise to the imaginal tissues. Externally the midgut is covered by a fine



Text-fig. 2. Larval gut of *Lyctus*. A, transverse section of proventriculus (× 100). B, longitudinal section of midgut (× 430). C, transverse section of hindgut through glandular bands (× 120). D, transverse section of hindgut through paired sacs (× 120). *B*, peritoneal membrane; *C*, chitinous lining; *E*, epithelium; *G*, gland cells; *H*, hyaline striated area; *I*, interstitial cell; *L*, lumen of gut; *L.S.*, lumen of sac; *M*, Malpighian tube; *Mc*, circular muscle layer; *Mm*, membrane binding Malpighian tubes to hindgut; *N*, nucleus of epithelial cell; *P*, cilia-like processes; *S*, seta.

peritoneal membrane and seems to be devoid of any muscle layers: progress of the gut contents must presumably be brought about by general body movement.

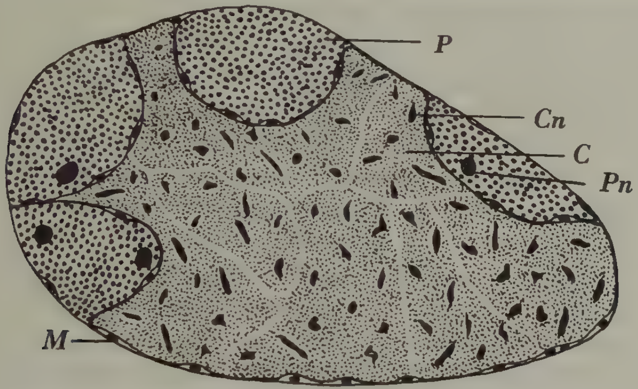
At the junction of midgut and hindgut arise six green or brown Malpighian tubules, the distal ends of which are closely bound to the hindgut about half-way along its length (Text-fig. 2C). This condition is present in a number of insects and is probably concerned with water economy when little is present in the food (Wigglesworth (34)). Insects living in seasoned timber cannot afford to lose water by excretion.

The hindgut is folded into a loop in the body cavity during the course of which the wall, consisting of muscles, epithelium and chitinous lining, shows extraordinary variation in folding and relative thickness of the different layers. The wall of the first part is thrown into six or seven longitudinal folds, and the cavity has a chitinous lining very densely covered with fine bristles which probably prevent solid particles re-entering the midgut. Soon after its commencement, the hindgut runs horizontally across the body, and each end of the loop enters into close contact with the atrium of the corresponding large spiracle on the eighth abdominal segment. Closely applied to this region of the gut, but apparently not opening into it, are two sacs (Text-fig. 2D) lined with chitin which unite with each other at both ends. The posterior descending part of the rectum also comes into close contact with these sacs. On the side next to the sacs the gut wall is formed of irregular epithelial cells showing few nuclei and a thick internal lining of chitin which occupies up to one-half of the lumen. On the opposite side, the wall has an external layer of circular muscle fibres, a thin folded epithelium, and a delicate chitinous intima closely pressed to the epithelium surface. It is not known whether this structure has any special function. Two bands of glandular cells (Text-fig. 2C) are present on opposite sides of the hindgut about half-way along its length: their prominence varies considerably in different larvae, and it is possible that they function in absorbing water from the frass, which is always dry by the time it reaches the hind part of the rectum. Posteriorly, the hindgut is characterised by the well-developed sheath of circular muscles and the thick, folded chitinous lining, the surface of which is channelled longitudinally by numerous fine, shallow grooves.

From a histological study, it is clear that digestion must occur principally in the midgut, but is no doubt continued whilst the food is passing through at least the anterior portion of the hindgut, the function of which is probably chiefly that of absorption. The digestive enzymes are secreted mainly, if not entirely, by the walls of the midgut, since there are no caeca, salivary or other glands appended to the gut, except the small bands of glandular tissue on the hindgut.

THE ROLE OF MICRO-ORGANISMS IN RELATION TO DIGESTION

The presence of micro-organisms within the body of *Lyctus* (larva, pupa and adult) was first reported by Gambetta (12), whose paper I have unfortunately been unable to consult; his findings have, however, been summarised by Buchner (3). Kojima (17) has confirmed Gambetta's observations during his study of the larva of *L. linearis*. I have examined the so-called mycetomes in order to form an opinion upon the existence of symbiosis and the possibility of its relation to digestion, but my observations do not entirely agree with those of the two workers quoted.



Text-fig. 3. Longitudinal section of *Lyctus* larval mycetome. *C*, central syncytium with nuclei *Cn*; *M*, membrane; *P*, peripheral syncytium with nuclei *Pn*. (×200).

In *Lyctus* larvae, a pair of organs (mycetomes) lie free in the haemocoelic cavity surrounded by the fat body. They are not connected with the gut nor any other organ, although they always lie in approximately the same position, namely, dorsally at the posterior end of the midgut. When dissected out, the structures are seen to be ovoid in shape and transparent, slightly less transparent in the centre than round the margin. If sectioned and stained, each mycetome (Text-fig. 3) is found to consist of two parts, one peripheral and the other central. The whole structure is enclosed in a thin membrane within which can be seen 6–12 peripheral groups of darkly staining bodies forming a ring round the central portion, but not covering it completely. These groups are separated from each other and from the central portion of the mycetome by a second fine membrane. Each peripheral group is a synctium containing 3–6 rounded nuclei which are not easily seen, and a large number of very small bodies so densely packed that it is extremely difficult to determine their structure. They appear to be oval with a somewhat irregular margin, and each contains two or three basophile inclusions. The central portion of the mycetome is also a syncytium, the nuclei of the component cells being hypertrophied and very irregular in outline. The cytoplasm is extremely densely packed with very minute, cylindrical, bacillus-like bodies which have a darkly staining spot at one end. The large oval symbionts, which Gambetta and Kojima describe in the central portion of the mycetome, must correspond to what I regard as nuclei. In sections, the central portion is very clearly divided into five or six parts, but this is probably to some extent due to shrinkage during fixation.

The mycetomes persist unchanged in the pupa, but take up a position ventrally at the anterior end of the abdomen. I have not investigated their occurrence in the adult nor the mode of transmission, but have distinguished miniature mycetomes in sections of newly hatched larvae. According to Buchner, Gambetta states that the symbionts break out of the mycetomes and penetrate into the ovarioles, thus infecting the eggs.

Among wood-borers only the Bostrychidae (Mansour (19)) have mycetomes in the larva resembling those of the Lyctidae, whilst, among the Coleoptera not living in wood, some Cucujidae (Koch (16)) and Curculionidae (Buchner (3), Mansour (20)) bear the greatest resemblance. There is no evidence to show whether or not the mycetomes of *Lyctus* contain organisms which are symbiotic. As there is no connection with the gut, it is evident, however, that, if the organisms are symbiotic, they can play no part in digestion by the host, unless they act as a source of vitamins which are lacking in the food, a possibility suggested by Wigglesworth (34) to account for the presence of similar mycetomes in other insects.

INVESTIGATION OF THE FOOD REQUIREMENTS OF *LYCTUS* LARVAE

There are four methods by which the substances utilised for food by *Lyctus* larvae have been investigated, namely:

(i) Chemical analysis of food and excrement.
(ii) Removal of certain substances from the wood by extraction with solvents.
(iii) Testing for the presence of certain enzymes in the gut.
(iv) Feeding on artificial diets.

Each of the above methods has yielded information which helps towards solving the problem, but, as knowledge of the chemistry of wood is as yet incomplete, particularly with reference to the cell contents, it is difficult to correlate the results of the various lines of investigation.

Chemical analysis

Campbell (5) published in 1928 the results of analyses of sound oak sapwood and *Lyctus* frass which showed that there was no change in the proportions of the main cell wall constituents of wood after passing through the digestive tract of the larvae. He concludes that either a portion of the wood is digested completely or *Lyctus* larvae feed only upon the cell contents. As it is most unlikely that a larva could digest completely so resistant a substance as wood, it would appear that the cell contents only are available as food. Campbell (6) has recently confirmed this by showing that there is a considerable reduction in starch content during the passage of wood through the larva. If the larval bore-dust is tested with iodine-potassium iodide solution, starch grains can be detected. This shows either that *Lyctus* larvae do not ingest all the wood particles which they bite off or that a number of starch grains pass through the gut unaltered, being in some way protected from the action of the digestive ferments.

For comparison with *Lyctus*, the food (oak) and frass of larvae of *Xestobium rufovillosum* were also examined, and here a definite drop in the cellulose and pentosan content was observed, showing that *Xestobium* larvae can utilise as food part of the wood substance itself. Ripper (27) has performed a similar analysis with maple as the food material, and has found that a considerable loss in cellulose content of the food occurs during digestion by *Xestobium* larvae.

Extraction of wood with solvents

Boodle and Dallimore (2) have concluded that attack by the Bostrychid beetle, *Dinoderus minutus*, in bamboo is governed by the amount of soluble sugars in the wood, and they found that storing bamboo under water for three months, a treatment commonly adopted in India, greatly reduced the soluble sugar content and rendered the wood unattractive to the beetles, although starch was present in considerable quantity. Snyder (30) says that losses due to *Lyctus* beetles can be prevented "...by submergence in water for four months, which renders the wood immune from attack, even after removal from the water".

In order to investigate the effect of leaching, a preliminary experiment was started in 1930 when several pieces of air-dried oak sapwood were soaked in cold water until the extract was no longer coloured yellow. The samples were then air-dried again and exposed to attack by *Lyctus* beetles along with an untreated control specimen, and monthly observations were made of the larval activity. Of the seven pieces of leached sapwood only one showed signs after 12 months of active infestation, indicated by a single small pile of wood dust on the surface, whereas the control was heavily attacked. Two samples were cut up and examined in detail and it was found that numerous eggs had been laid in the vessels, but the young larvae had died after some 3½ months, during which time they tunnelled only a short distance in the wood.

It was evident that leaching with water had an adverse effect on the development of *Lyctus* larvae in the treated oak sapwood, and it was therefore decided to investigate the problem in greater detail in 1931. Small samples, 1½ × ½ × ½ in., were extracted with water, alcohol or ether in a Soxhlet apparatus by the Wood Chemistry Section of the laboratory until the extract was colourless. The alcohol- and ether-extracted samples were kept in a vacuum desiccator until the solvent had been removed, and all specimens were then placed in another desiccator over sulphuric acid diluted to give a relative humidity of 75 per cent. to the enclosed air: the wood assumed an equilibrium moisture content of about 15 per cent. Owing to an accident some sulphuric acid was spilt over all but three of the samples, one water-, one alcohol- and one ether-extracted. These were exposed to attack by *L. brunneus* with a few of the less badly contaminated pieces: a new set of specimens from a different source of oak sapwood was similarly prepared, extracted and exposed to attack by beetles of the same species. The samples of both series were observed periodically for external signs of larval activity, until they were examined in detail by complete cutting up some 7–9 months after exposure to attack.

It was found that the acid-contaminated samples contained living larvae which were undersized, presumably through restriction in the amount of wood available as food. In the three specimens which were not touched by acid, and in a control sample of normal oak sapwood exposed to attack with them, live larvae were also found, but, whilst in the alcohol- and ether-extracted and control samples the larvae were 2–3 mm. in length, those in the water-extracted sapwood had attained a length of only 0·5–1 mm., that is, were scarcely larger than first-stage larvae. Thus, extraction of the wood with alcohol or ether had no apparent effect upon the development of *Lyctus* larvae, but extraction with water inhibited their development very considerably.

Of the thirty samples comprising the second series, it was found that only four showed signs of active infestation. These four specimens were all controls, but five other control pieces did not show signs of active attack. The extracted samples contained a number of larvae, all of which had died after tunnelling a few millimetres. Comparison of the two sets of results suggested that, with the exception of the four attacked controls of the second series, there was a radical difference between the wood used for the two series of experiments. Light was thrown upon the problem by an examination, carried out in conjunction with Dr S. E. Wilson (35), of samples of ash, some of which he had rendered free from starch and submitted to this laboratory for exposure to infestation by *L. brunneus*. The starch-free ash was not damaged by *Lyctus*, whereas that containing starch was severely attacked. The samples used in the extraction experiments were therefore treated with iodine-potassium iodide solution and examined for starch content under a low-powered binocular microscope. The wood of the first series showed abundance of starch grains, whereas that of the second series proved to be starch-free except for the four control samples which had been successfully attacked. In the twenty-one starch-free samples which had been extracted, 363 tunnels of young larvae were discovered, but none of these larvae had lived more than a month or two.

Two factors had now appeared which could influence the development of *Lyctus* larvae: the presence or absence of a water-soluble substance and the quantity of starch in the wood.

In 1932 it was decided to repeat and extend some of the previous year's experiments in view of the importance of starch. The experimental procedure was the same, but special care was taken to obtain material containing starch by using the sapwood of a freshly cut, winter-felled oak log. Alcohol extraction was omitted. A number of specimens of starch-free oak sapwood were obtained from another source for comparison with the starchy samples. Monthly observations were made for any external signs of activity, and the final results obtained when the samples were cut up 7–9 months after the start of the work are summarised in Table I.

These results confirm the earlier conclusions that extraction of oak sapwood with ether does not in any way hinder the development of *Lyctus* larvae, whereas extraction with warm water slows down the growth very greatly and causes a high mortality of young larvae. Furthermore, it is quite clear that larvae are unable to develop in samples lacking starch. An attempt to introduce starch into wood which was naturally starch-

Table I

Results of 1932 *extraction experiments*

No. of samples	Amount of starch (see Pl. XVI, fig. 2)	Extracting liquid	Subsequent treatment	Results	Remarks
8	Moderate	Ether	—	All samples contain living larvae 1·5–4 mm. long	Majority of larvae over 3 mm. long
8	Moderate to abundant	Water	—	5 samples contain living larvae 0·75–1·5 mm. long and many minute dead larvae	3 samples contain no eggs nor larvae
14	Little to moderate	—	—	All samples contain living larvae or pupae. Larvae 1·5–3·5 mm. long	The small larvae occurred in samples having only a little starch present
2	Abundant	Water	Soaked in cold 2% starch solution	Live larvae present, 0·75–1 mm. long	Only a little starch had been adsorbed on the walls of the larger vessels
2	Little to moderate	—	Do.	Live larvae present, 1–2 mm. long	Do.
7	None	—	—	All samples contain minute, dead larvae only	
5	None	Water	—	All samples contain minute, dead larvae; 1 living larva, 2 mm. long, found	One sample had a single medullary ray containing abundant starch in which the live larva was tunnelling
6	None	—	Soaked in cold 2% starch solution	All samples contain minute, dead larvae only	Only a little starch had been adsorbed on the walls of the larger vessels

free, by soaking in soluble starch solution, did not succeed, since, on testing with iodine solution, it was found that a slight blue coloration was visible only where starch had been adsorbed on the walls of the large vessels. The amount of starch thus introduced was not sufficient to influence larval growth. For the same reason, soaking starch-containing samples in starch solution gave rise to no increase in the rate of larval development. It will be seen from the table that there was considerable variation in size of the larvae cut out of the samples, and this is probably to be accounted for by individual differences in normal growth rate and by overcrowding in the small specimens used. Such differences in larval size are often seen in large samples of wood which have been heavily infested.

During 1933 further experiments were started to establish finally that starch forms one of the principal food substances of *Lyctus* larvae. All specimens were cut from the same board of oak sapwood, which was shown to contain plenty of starch. A number of samples were extracted in a beaker with distilled water at 60° C. The water was changed frequently until no more colouring matter was dissolved out. During the first day the yellow extract reduced Fehling's solution with the production of a definite red precipitate: by the third day the extract was colourless and no longer contained reducing substances. The 60° C. extract showed no marked colour change on addition of iodine-potassium iodide solution. Half of the samples were then removed and dried, the remainder being further extracted with boiling water. The liquid again became yellow, did not reduce Fehling's solution, but gave a deep blue coloration with iodine solution, showing that starch had been removed from the wood. Boiling was continued for a week, when the extract no longer gave the blue colour with iodine. The samples, when dried and tested, showed that practically all of the starch had been boiled out. Extracted samples and untreated controls were exposed to attack by *L. brunneus* beetles and examined monthly, as before, in order to note the progress of attack, so far as could be seen externally. All signs of activity ceased in the samples extracted at 100° C. after 1–2 months; a small amount of bore-dust still appeared on samples extracted at 60° C. after 10 months, whilst the controls all showed considerable quantities of dust after the same period. The samples were cut up for detailed examination 10 months after the start of the experiment and the results are shown in Table II.

The results in Table II show that *Lyctus* larvae cannot live in wood extracted with boiling water and thus artificially rendered starch-free, whereas in wood extracted with water at 60° C. larvae find just sufficient nourishment for life but insufficient for normal growth.

It will be noticed that the size of the living larvae was somewhat smaller than in the 1932 work: this is possibly due to the very dry summer and winter 1933–4. The moisture content was determined of part of one sample from each of the three series mentioned in Table II and found to be 9·5, 8·5 and 9·4 per cent. respectively. Fisher (10) states that *Lyctus* cannot live in wood of a moisture content below 8 per cent., so that the rate of growth would probably be somewhat retarded at the moisture contents found.

Table II

Results of 1933 *extraction experiments*

No. of samples	Extracting liquid	Amount of starch	Results
9	Water at 60° C.	Abundant	Live larvae 0·5–1·0 mm. long present; majority about 0·5 mm.
9	Water at 100° C.	None (after extraction)	Only minute dead larvae present
9	—	Moderate to abundant	Live larvae 0·5–2·5 mm. long present; majority about 1·5 mm.

No precise information has been obtained upon the composition of the constituent which is insoluble in ether but easily removed from the wood by extraction of small samples with water at 60° C. and the absence of which slows the rate of larval development. The aqueous extract contains tannins and probably some sugars, since Fehling's solution is reduced strongly by the first day's extract. It is also possible that nitrogenous materials, *e.g.* proteins, or even vitamins are removed, but it is not possible to say which, if any, of the substances mentioned are necessary for normal larval growth.

Digestive enzymes of the larval gut

Testing of tissue suspensions of the larval gut, including the digestive juices, was greatly handicapped by the small size of *Lyctus* larvae, the length of those used being between 2 and 5 mm. A large number of larvae had, therefore, to be dissected in order to obtain a sufficient quantity of extract of a reasonable concentration. Larvae were cut out of infested wood the day before they were required for dissection, since all the dissections had to be undertaken in one day in order that the suspension should be as fresh as possible and bacterial action kept at a minimum before the whole preparation was preserved with toluene. It is impossible to say what species of larvae were used in this work, but judging by the material employed there is little doubt that at least two species were involved, namely *L. brunneus* and *L. planicollis*.

The technique of this investigation was based on that of Swingle (31) and 100–200 larvae were dissected for each series of experiments. The digestive organs, freed so far as possible from the fat body, were collected in 3–4 c.c. of recently boiled distilled water and ground with sand by means of a small agate pestle and mortar. The sand had previously been purified by washing several times in distilled water, burning in a muffle furnace for an hour to remove any organic materials, and then receiving a final wash. After grinding, the sand was allowed to settle and the supernatant liquid pipetted off; more water was added and the grinding repeated until about 50 c.c. of tissue suspension had been obtained. A layer of toluene was added as a preservative and the larger particles in the suspension allowed to settle out. This tissue suspension, containing the gut enzymes in dilute solution, was mixed with various substrates in test-tubes, the mixture being covered with a layer of toluene and incubated at 30° C. for 4–5 days. The tubes were plugged with cotton-wool to prevent evaporation of the preservative. After incubation the mixtures were tested for breakdown products of the substrate caused by enzymic activity. A second series of tubes containing boiled tissue suspension plus substrate was set up as controls. The double series of experiments was repeated three times with different batches of larvae.

In testing for enzymes acting upon carbohydrates, tissue suspension was mixed with a 1 per cent. aqueous solution of the substrate or, in the case of insoluble materials, with a small quantity (about 0·02 gm.) of the solid substance. After the incubation period the mixtures were boiled with Fehling's solution to test for the presence of reducing sugars or, if the original substrate reduced Fehling's, with Barfoed's reagent for the detection of monosaccharides.

The results of one series of experiments are shown in Table III.

Two further series of tests confirmed the results given in the table except that no definite indication of the presence of maltase was obtained. An additional test for this ferment was, however, carried out with a more concentrated tissue suspension and a positive result then again obtained. It is surprising that difficulty should have been experienced in detecting maltase in view of the presence of amylase either in relatively large amount or in a very active form, but it is possible that the experimental conditions were not correct for the optimal action of maltase.

The enzymes have been named in Table III according to the substrata upon which they act. It is improbable, however, that the specific enzyme lactase would exist in the larval gut of *Lyctus*, since lactose is not a sugar which occurs in the plant kingdom. It seems more likely that there is in the gut an enzyme capable of hydrolysing to monosaccharides certain disaccharides which are related in chemical constitution.

Table III

Tests for carbohydrate enzymes of Lyctus *larvae*

(No. of larvae used = 205)

Exp. no.	Enzyme	Amount of suspension c.c.	Substrate	Amount of substrate c.c.	Test	Result	Remarks
1	Control	1	1% Starch	3	Fehling's	–	No precipitate
1*a*	Amylase	2	,,	5	,,	+ + +	Very heavy ppt.
1*b*	Amylase	1	0·1% Starch	3	I–KI	+ + +	No blue colour after 11 days
2	Control	1	1% Sucrose	3	Fehling's	–	No ppt.
2*a*	Invertase	2	,,	3	,,	+ + +	Very heavy ppt.
3	Control	1	1% Maltose	3	Barfoed's	–	Very slight ppt.
3*a*	Maltase	2	,,	3	,,	+ +	Heavy ppt.
4	Control	1	1% Lactose	3	,,	–	No ppt.
4*a*	Lactase	2	,,	3	,,	+ + +	Very heavy ppt.
5	Control	3	Cellulose*	—	Fehling's	–	No ppt.
5*a*	Cellulase	3	,,	—	,,	–	,,
6	Control	3	α-Cellulose*	—	,,	–	,,
6*a*	α-Cellulase	3	,,	—	,,	–	,,
7	Control	3	Hemicellulose A*	—	,,	–	,,
7*a*	Hemicellulase	3	,,	—	,,	–	,,

* Prepared from oak sapwood by Wood Chemistry Section, F.P.R.L.

In addition to tests with the carbohydrates mentioned in Table III, the action of the digestive enzymes was tried upon "starch" isolated from oak sapwood by the Wood Chemistry Section of this laboratory (6). The hydrolysis was nearly as rapid and extensive as that obtained with soluble starch.

For pepsin and trypsin Swingle's (31) colour release method was tried. Fibrin was stained in aqueous congo red and washed with hydrochloric acid (*p*H 3·0) until the acid remained colourless. Stained fibrin, 2 c.c. of tissue suspension and 3 c.c. hydrochloric acid (*p*H 3·0) were then mixed and incubated when any digestion of the fibrin by pepsin should release stain into the surrounding liquid. Aniline blue, recommended by Swingle as a fibrin stain in testing for trypsin in slightly alkaline solution, was found to be unsuitable owing to its continuous slow leaching out in sodium bicarbonate solution (pH 7·6): borax carmine was therefore substituted. Erepsin was tested for by mixing a few drops of brom-thymol blue indicator with 5 c.c. of 4 per cent. peptone solution, adding 2 c.c. of tissue suspension and then dilute sodium bicarbonate solution until the indicator turned blue. If erepsin is present the amino acids liberated from the peptone during incubation should cause the colour to change from blue to green or yellow. Finally, to detect the presence of a lipase, 2 c.c. of tissue suspension were coloured blue with brom-thymol blue and a drop of dilute sodium bicarbonate solution; 1 c.c. of olive oil was added and the mixture shaken. A lipase, if present, should release fatty acids from the oil, causing the lower layer of liquid to become green or yellow.

No clear indication of the presence of proteinases or lipase was obtained, however, possibly because of the great dilution of the tissue suspension. Schaede's (28) photographic plate method for the detection of proteolytic enzymes in plants was therefore tried, but this author does not give details for the preparation of the plates and much preliminary work had to be undertaken before the following procedure was adopted. An Ilford screened chromatic 270 H and D plate, $6\frac{1}{2} \times 4\frac{3}{4}$ in., was exposed for $\frac{1}{2}$ sec. in a printing frame at a distance of 18 in. from a ground-glass screen illuminated behind by a 60-watt lamp. Development was carried out in standard metol hydroquinone and followed by fixation in acid hypo. The plate was very thoroughly washed for some hours in running water, then dried and out into six strips each 1 in. wide.

During preliminary work a culture of *Bacillus liquefasciens fluorescens* was used as a source of protein enzymes. The original culture was kindly supplied by Dr S. E. Jacobs, Department of Bacteriology, Royal College of Science, South Kensington, S.W. 7, and was subcultured on slopes of nutrient gelatin of the following formula:

Meat extract	2 gm.
Sodium chloride	5 ,,
Peptone	10 ,,
Gelatin	150 ,,
Water	1000 c.c.

The slopes were incubated for 4–5 days at 24° C. until most of the gelatin was liquefied. A strip of photographic plate was inoculated with alternate drops of sterile water and liquefied gelatin from a culture and incubated at 24° C. in a desiccator containing water to prevent evaporation of the inoculum. After 48 hours the strip was washed with cold running water.

The black film was completely dissolved from those spots inoculated with bacteria, whereas no lightening in density was apparent where drops of sterile water had been placed.

When testing for proteinases in *Lyctus*, the guts of forty larvae were dissected out and torn up as finely as possible with needles in about 0·75 c.c. of sterile water. One-half of this quantity was then heated in a boiling water-bath for ½ hour in order to inactivate any enzymes present. Drops of the suspension, sterile water and boiled suspension were placed alternately on strips of plate. After 24 hours' incubation in the moist desiccator, the strips were washed in cold running water when the gelatin acted upon by the gut enzymes was dissolved away completely; the water and boiled suspension spots showed no change.

In order to test for the action of bacteria in the larval gut suspension, a similar experiment was carried out but each drop of the different materials on the strip was covered with toluene. The result was the same as before. As a further check, tubes of malt agar were inoculated with normal gut suspension and with boiled suspension. Examination after 3 days' incubation at 24° C. revealed that two slopes inoculated with normal suspension showed a total of twelve very small mould colonies and one minute white bacterial or yeast colony, whereas two boiled suspension slopes had no signs of any growths. It is highly improbable, therefore, that fungal or bacterial action could have been sufficient to account for the results of the enzyme tests. Later experiments showed that the proteolytic action of the larval digestive juice could be clearly demonstrated by incubation drops of a suspension containing 4–6 larval guts, torn up in 0·25 c.c. of sterile water, on a strip of blackened plate for 10 min. at 30° C. and washing as before.

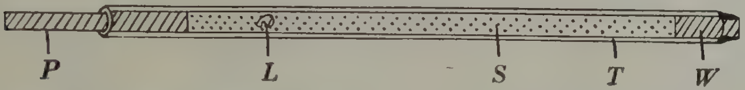
It may be concluded that *Lyctus* larvae possess a ferment capable of breaking down proteins, but the experiments give no clue to the identity of the enzyme.

It is clear from these experiments that the gut of the *Lyctus* larva is fitted to digest proteins, starch and certain disaccharides occurring as cell contents in wood, but is unable to utilise the more complex carbohydrates such as cellulose and hemicellulose which are present as constituents of the cell wall. This is in accordance with the results previously obtained from chemical analysis of food and frass and from the extraction experiments, but throws no light on the problem of what substance or substances, removed from wood by water at 60° C., are so necessary for normal larval development.

Relatively little work has yet been carried out upon the hydrogen-ion concentration of the gut contents of insects, but Swingle (32) has shown that, among wood-borers, the midgut contents of a *Parandra brunnea* beetle (Cerambycidae) had a $p\text{H}=6{\cdot}18$, and in the larva of another Cerambycid, species unknown, the foregut (pH 6·57) and midgut (pH 6·93) are very slightly acid, whereas the hindgut (pH 8·12) is definitely alkaline; in the foregut of the carpenter ant (*Camponotus herculeanus*) the very high value of pH 4·00 was obtained. In the powder-post beetles, the hydrogen-ion concentration of the tissue suspension of the larval gut was determined colorimetrically as pH 6·5, indicating that the digestive juices are slightly acid. Since the extract was made from the whole gut, however, it is possible that the values might deviate considerably in different parts from this mean value.

Artificial diet

It is extremely difficult to make larvae of *Lyctus* feed upon a powdered substance, since, once the support of the tunnel wall is removed, they seem unable to move or feed freely and, as a result, soon die. Several methods were tried unsuccessfully before a technique was discovered which promised comparable results with different substrates. Glass tubing, 3 mm. in internal diameter, was cut into 8 cm. lengths (Text-fig. 4), and one end of each piece closed with a short wooden plug and then sealed with paraffin wax. The powdered substrate (passing a 60-mesh screen) was introduced in small quantities and packed tightly by means of a length of glass or metal rod which just fitted the cavity of the tube. About 2 cm. from the top a larva, 1·5–2 mm. long, was inserted; a further 1 cm. was carefully packed with substrate and the tube closed with a second wooden plug projecting at least 1 cm. from the glass and not waxed. A number of tubes, each containing one larva, were thus started with each substrate and kept in an incubator at 24° C. with a relative humidity about 70 per cent. Examination under a binocular microscope once a week enabled the progress of the larvae to be watched. As each larva burrowed through the powdered food material, which was thus reduced to a smaller particle size and consequently packed more tightly, it formed for itself a small cell and tended to lose support for



Text-fig. 4. Tube for feeding *Lyctus* larvae on powdered substrates. *L*, larva; *P*, unwaxed plug; *S*, substrate; *T*, glass tube; *W*, waxed plug.

feeding. The unwaxed plug was therefore periodically pressed further into the tube, and the substrate once more compressed round the larva.

The substrates used and the results of the experiments expressed as average length of life of the larvae are given in Table IV. Sapwood only was used in these experiments; the signs (+) and (−) following the names of timbers indicate respectively that starch was present in or absent from the sapwood. The figures in the column headed "Substrate" refer to parts by weight of the constituents in mixtures. All larvae surviving their first moult, with the exception of a few accidentally killed by squashing when adjusting the unwaxed plug, are included in the calculations of the average length of life.

Table IV

Duration of life of Lyctus *larvae feeding upon various diets*

Serial letter	Substrate	No. of larvae used	Av. length of life in days	Remarks
A	Control without food	9	48·8	
B	Norway spruce (−)	16	53·5	
C	Beech (−)	15	52·2	
D	4 Beech (−) + 1 peptone	6	54·0	
E	4 Beech (−) + 1 "starch" extracted from oak	12	81·8	
F	4 Beech (−) + 1 soluble starch	8	232·8	
G	Oak (+)	11	81·7	
H	4 Oak (+) + 1 peptone	9	116·3	
I	4 Oak (+) + 1 soluble starch	7	253·2(5)	1 pupated after 131 days; 1 alive after 402 days (28. x. 35)
J	Oak extracted with water at 60° C. (+)	10	60·8	
K	4 Oak extracted with (+) + 1 sucrose	6	280·0(3)	3 alive after 395 days (28. x. 35)
L	4 Oak extracted with (+) + 1 glucose	6	?	4 accidentally killed; 2 alive after 395 days (28. x. 35)
M	Soluble starch	7	155·7	
N	"Starch" extracted from oak	6	66·7	
O	4 Soluble starch + 1 sucrose + 1 peptone	6	262·0	1 killed accidentally after 221 days; 2 pupated after 322 and 353 days respectively

It will be seen that larvae confined on the starch-free wood flour of Norway spruce (series B) or beech (C) live very little longer than the controls without food (A), and that the addition of peptone, as a source of nitrogen, to the beech (D) makes no appreciable difference. Addition of "starch" extracted from oak sapwood to starch-free beech (E) increases the length of life by more than one-half, whilst larvae can live for 8 months when soluble starch is mixed with the beech flour (F). These experiments with beech indicate that peptone cannot increase the length of life of the larvae in the absence of suitable carbohydrates, and that the addition of starch provides much of the necessary carbohydrate requirement.

In normal oak sapwood (G) the larvae lived on an average 81·7 days, and the failure to live and complete their development can be ascribed only to the experimental conditions. It would appear, therefore, that any substrate which supports larvae for more than an average of 81·7 days must include some constituent which has good nutritional value, thereby reducing to some extent the need for continuous tunnelling under unfavourable conditions. Peptone added to the oak flour (H) increases the length of larval life considerably and must therefore act as a source of food. The admixture of soluble starch with the oak (I) enables the larvae to live for 9 months, and one larva which was slightly longer than 2 mm. when inserted in the tube pupated after 131 days and eventually turned into an undersized beetle. Oak sapwood flour (J), treated with water at 60° C. in the same way as the small blocks in the 1933 extraction experiments, supported the young larvae for 60·8 days, a figure well below that for the unextracted material (G), thereby confirming the fact that water at 60° C. will remove from the wood, without any apparent reduction in the starch content, some substance which is necessary for larval development. The addition of sucrose (K) or glucose (L) to water-extracted oak enables the larvae to live and grow slightly for more than 12 months, and this strongly suggests that sugars are the water-soluble constituents of wood without which normal larval development cannot proceed.

Larvae confined on soluble starch (M) lived a considerable length of time, but never increased in size, showing that whilst starch is perhaps the principal food of *Lyctus* larvae it is, in itself, insufficient to satisfy the requirements of the insects. Young larvae lived an average of 66·7 days on "starch" extracted from oak sapwood (N), but it is possible that the treatments undergone during extraction from the wood alter the "starch" in some way, thus rendering it more difficult to assimilate. This is also suggested by comparison of series E and F. The last series (O) comprised some larvae which were placed in a mixture of starch, sucrose and peptone. The mixture was slightly hygroscopic and became rather sticky, but, in spite of this, the larvae grew steadily and showed every sign of being quite normal and healthy. Two larvae pupated successfully and turned into normal adults. Since the larvae tunnelled very little, the diet must have had great nutritive value; furthermore, the mixture was completely digested, for excrement was almost entirely absent.

The results of this series of experiments confirm that starch is a necessary constituent of the food, and indicate that a soluble sugar, such as sucrose or glucose, and probably a small amount of protein, are also required by *Lyctus* larvae. None of these substances, however, is a complete food by itself. The series O experiments prove conclusively that *Lyctus* larvae can satisfy their food requirements with the cell contents of suitable timber and do not need any additional nutriment which may be available in the cell-wall substance.

Selection of wood for oviposition

It has already been shown (26) that vessel diameter is a factor governing the liability of different species of wood to attack by *Lyctus*, but the present investigation has clearly demonstrated that it is a matter of great importance to the insect that the females should be able to detect wood containing quantities of food sufficient for normal development. It was evident from the results of the extraction experiments that relatively few eggs were laid in samples which were starch-free compared with untreated controls containing starch. Exceptional instances were noted, however, in which a starch-free sample contained a large number of dead larvae, and the only explanation that can be offered is that the physiological urge in the females to lay eggs must, in these cases, have overcome their disinclination to oviposit in unsuitable wood. Females have on rare occasions deposited eggs in a glass tube or Petri dish when no wood was present.

A special experiment based on the knowledge that water-extracted and starch-free samples are unsuitable for larval development, was undertaken to test the ability of *Lyctus* beetles to detect the suitability of wood for oviposition. Thirty samples, each $1 \times \frac{1}{2} \times \frac{1}{2}$ in., were cut from English oak sapwood containing abundant starch. One series of ten samples was used as controls, a second series of ten was extracted with water at 60° C. until the extract was colourless, and the third was extracted with boiling water until the extract was colourless and starch-free. A fourth series of ten samples was cut from a piece of naturally starch-free oak sapwood. Two pairs (Nos. 1 and 2, 3 and 4) from each series were exposed separately in glass-topped tins to attack by *L. brunneus*, eight pairs of beetles to each pair of samples. The remaining twenty-four samples (Nos. 5–10 of each series) were placed in a large tin in such a way that no two similar samples lay side by side, and thirty-six pairs of *L. brunneus* were added. Since the eggs of this species normally hatch in 8–9 days at room temperature, the samples were removed after 7 days and, if not cut up immediately, were exposed to a temperature of 60° C. for 1 hour in order to kill all the eggs present. After this treatment the samples could be examined when desired. The number of eggs laid in each piece was determined by shaving away the wood with a razor and counting the eggs, as they were exposed, with the aid of a binocular microscope. In addition to the eggs, the number of so-called "feeding marks" (Plate XVI, fig. 1) on the surface of each sample was recorded. These marks have been previously described by Fisher (10) and are made by beetles gnawing short, narrow incisions on the surface of the wood: as shown later, they are not really "feeding" marks but more in the nature of "tasting" marks and, for want of a better term, will in future be referred to by this name.

In Table V is given the number of eggs and tasting marks for each sample, with averages according to whether samples were exposed to attack in pairs or grouped together.

Table V

Detection of suitability of wood by ovipositing Lyctus *beetles*

	Eggs				Tasting marks			
Sample no.	C(+)	W(+)	BW(−)	SF(−)	C(+)	W(+)	BW(−)	SF(−)
1	87	78	1	3	48	45	8	1
2	54	45	17	0	33	41	4	0
3	71	39	5	21	42	41	3	3
4	87	95	0	56	60	55	3	15
Average for samples in pairs	74·8	64·3	5·8	20·0	45·8	45·5	4·5	4·8
5	101	0	1	11	52	31	9	1
6	60	0	0	1	48	21	9	3
7	105	0	0	1	46	22	10	1
8	46	4	0	0	51	20	3	4
9	101	15	0	0	55	40	8	2
10	75	2	4	0	44	18	8	3
Average for grouped samples	81·3	3·5	0·8	2·2	49·3	25·3	7·8	2·3

C = untreated controls.
BW = extracted with water at 100° C.
SF = naturally starch-free.
W = extracted with water at 60° C.
(+) = containing starch.
(−) = starch-free.

If we consider first the number of eggs laid in the various samples, we find that for samples 1–4 there is no significant difference between the means of the C and W series, whereas the means of the BW and SF series are considerably lower. It is evident from these figures that, when beetles are restricted in their choice of wood for oviposition, they will lay equally well in samples water-leached at 60° C. and in untreated controls, but are disinclined to lay in the boiled and naturally starch-free samples. The values obtained for the two pairs of SF samples are greatly divergent, and this can be accounted for only in the same way as the variation encountered in the extraction experiments (see p. 390). Where the beetles were given a choice of twenty-four samples the number of eggs laid in the controls was large as before, but in the W, BW, and SF series was very small. The beetles can evidently determine before oviposition whether or not wood is suitable and, since the samples were similar but for their food value to larvae, this food value must be the factor governing the number of eggs laid in the various samples. It is very interesting to note that, whereas beetles will lay eggs freely when confined on wood extracted with water at 60° C., they tend to lay very few eggs in such wood when untreated samples are offered as an alternative. Boiling the wood in water removes some substance which must be present if normal oviposition is to take place, with the result that extremely few eggs are laid in BW samples whether exposed to the beetles alone or in combination with others.

Tasting marks show, in general, a distribution among the samples similar to that of the eggs. For samples exposed in pairs the average number of marks on C and W samples is high, whereas for BW and SF it is relatively very small. In the experiment with grouped samples the mean for the controls remains high and those for BW and SF relatively low, but the W mean assumes an intermediate value. The difference between the means of the C and W series for samples 6–10 is statistically significant, however, so that there is a definite reduction in the number of tasting marks on W samples when exposed to attack along with controls.

It is clear that there is some correlation between the number of tasting marks on a sample, the number of eggs laid therein, and the food value of the wood: it would seem, therefore, that the making of tasting marks is closely bound up with oviposition. Fisher (10) has suggested that these marks are made by beetles gnawing off small particles of wood for food, and has shown that they are made by the females only; this latter observation I have confirmed. Numerous dissections of male and female beetles, however, have failed to disclose in the adult gut any material resembling wood and the excrement is always fluid. It must be concluded, therefore, that the marks are not made by beetles seeking nourishment. The method of oviposition by these beetles in their natural habitat in the forest is not known, but the possibility must be considered that the marks are made in order to open vessels to admit the ovipositor; the nature of the marks, however, does not render it likely that they would be of much value for this purpose. Another possible explanation is that the marks are made by females to judge the nutritional value of the wood for larvae before egg-laying. Suitable wood usually shows numerous incisions, and it has been observed that a sample showing abundant starch at one end and little at the other has eggs and marks concentrated at the starchy end.

The fact that more marks are found on starchy than on starch-free wood, however, tends to contradict the assumption that the female tests the wood primarily by means of these incisions, since it might reasonably be expected that, once a female has tested a sample which is suitable, oviposition would proceed without further marks being made, whereas unsuitable wood would be tested many times in the hopes of finding a part which contained the necessary food substances. Furthermore, it is quite clear from the occurrence of starch-free samples containing no eggs and showing few or no tasting marks, that the beetles are able to detect the unsuitability of the wood without making these marks.

The facts can best be explained by assuming that the female beetles carry out a twofold chemotactic test to distinguish suitable from unsuitable wood. The results given in Table V suggest that the primary test, in which tasting marks are not made, is to detect the presence of starch. If this primary test shows the wood to be starch-free no further test is normally made, but if the wood is starchy some of the specimen is powdered by the mandibles in order to confirm the presence of starch or to test for the presence or absence of other necessary food materials, *e.g.* water-soluble constituents, since substances in a finely divided form always smell or taste more strongly. The marks would, therefore, seem to be "tasting" or "gustatory" rather than "feeding" marks and to be made secondarily after the exercise of an "olfactory" sense. In the case of samples extracted with water at 60° C., some difference (presumably the lack of sugars) between these and the control samples can be detected, but is not sufficiently important to restrict egg-laying if no other wood is available: on the other hand, if control samples are present, almost all of the eggs are laid in these.

A second set of experiments was undertaken to ascertain whether *Lyctus* beetles could appreciate differences in the amount of starch present in wood. The amount of starch was estimated as abundant, moderate, little or none by visual examination of the wood after treatment with iodine-potassium iodide solution (Plate XVI, fig. 2 A–D). Four samples of each category were prepared from oak sapwood as before, and two of each type were exposed as a separate pair to infestation by six pairs of *L. brunneus* beetles. The remaining eight samples were mixed and exposed in a common vessel to twelve pairs of beetles. The numbers of eggs and tasting marks were counted and the results are shown in Table VI. It must be pointed out that only the starch content of the samples was estimated, and nothing is known of the amount of sugars and protein present.

From numerous observations on the relation between intensity of *Lyctus* attack and amount of starch in wood made during the course of this work, it was assumed that the samples containing abundant or moderate quantities of starch would be suitable for larval development, whereas those classified as "little" or "none" would contain insufficient food for the larvae. This assumption is supported by the results given in Table VI, from which it will be seen that nearly all of the eggs were laid in the samples of the first two categories. It is evident, moreover, that beetles can not only differentiate between samples suitable or unsuitable for larval development, but also appreciate differences in the starch content of suitable samples and lay most of their eggs in those samples which contain the most starch.

Table VI

Detection by ovipositing Lyctus *beetles of differences in the amount of starch in wood*

Sample no.	Amount of starch	Number of eggs	Number of tasting marks
1A	Abundant	100	48
1B	,,	85	47
2A	Moderate	40	40
2B	,,	55	51
3A	Little	3	18
3B	,,	5	5
4A	None	2	2
4B	,,	12	16
5A	Abundant	154	47
5B	,,	164	60
5C	Moderate	25	28
5D	,,	27	60
5E	Little	0	3
5F	,,	3	2
5G	None	0	0
5H	,,	0	0

Reference to the tasting marks on the samples again shows that the number of these incisions is roughly correlated both with the food value of the wood and the number of eggs found, but gives no indication of the ability of the beetles to detect differences in the starch content of suitable samples.

The work so far carried out has not been sufficient to elucidate the exact significance of the tasting marks of *Lyctus* beetles in determining the nutritional value of wood. There can be little doubt that they are connected with the testing of the wood for food value, but they are not the primary test. In an attempt to gain more information on this primary test, some experiments have been undertaken in which various structures, e.g. maxillary palps, antennal club, etc., which might bear the chemotactic organs in the female beetle, were amputated. The beetles were then placed on samples of oak sapwood containing abundant or no starch and, after a given period, the number of eggs and tasting marks counted as before. The results, however, were inconclusive.

STARCH IN TIMBER

The starch content of living trees varies very much from species to species and according to the season of the year. Büsgen (4) has classified trees into two groups according to whether their reserve food materials are mainly in the form of starch or fat. In the starch trees, the starch grains occur either singly or in aggregates in the cells of the medullary rays and xylem parenchyma, and Haberlandt (13) has estimated that for many hardwoods the starch-bearing tissues form from 20 to 30 per cent. of the total volume of the sapwood. According to Schorger (29) the amount of starch does not usually exceed 3 per cent., but up to 5·9 per cent. has been recorded for elm. Mansour and Mansour-Bek (22) quote various authors to show that the starch content of different woods varies from 0 to 5·9 per cent. and the sugar content (calculated as glucose) from 0 to 6·2 per cent. The carbohydrates present as cell contents probably average 3–6 per cent.

Starch is generally at a maximum in the wood in the late autumn soon after the fall of the leaves and at a minimum in the early spring immediately following the opening of the buds. Normally it is confined to the sapwood, but in some tropical timbers, *e.g. Triplochiton scleroxylon* K. Schum. (Sterculiaceae), quantities of starch have been found in the heartwood as well as in the sapwood. Owing to the discontinuous distribution, no method has been found for determination of the amount of starch present except by visual estimation, following surface application of iodine-potassium iodide solution, on an arbitrary scale based upon the experience of numerous observations (Plate XVI, fig. 2 A–D). Wood chemists (6) have, so far, relied upon methods involving hydrolysis of the starch and subsequent estimation of the amount of glucose produced or extraction of the starch and gravimetric determination, but such procedures have the disadvantage that they necessitate destruction of all or part of the sample, and require a considerable time to complete one experiment. A simple, rapid and covenient means of determining the starch content of wood samples of small size would be very helpful, particularly in relation to work on the immunity starch level for *Lyctus*. "Immunity starch level" is a term used by Wilson (35) to indicate the amount of starch in wood which is just insufficient for the needs of *Lyctus* larvae. The determination of the immunity level, however, is complicated by the fact that the life cycle of *Lyctus* has been prolonged to two years, instead of the normal one, when wood containing a relatively small quantity of starch was exposed to attack: it may be possible to extend the period of development even beyond this.

Wilson has shown that the starch distribution in sawn timber of commercial sizes may be very irregular, and several cases of extraordinary distribution have come to light during the course of the present investigation. A good instance is illustrated in Plate XVI, fig. 3, where a portion of starch-bearing tissue has been occluded in the normal heartwood of oak and shows severe attack by *Lyctus*: this sample is exceptional in the extreme irregularity of its starch distribution. In many cases it is quite possible to form an opinion of the probable susceptibility of boards, etc., to attack by treating a few small newly cut areas with iodine solution. It is essential that the surface tested (preferably obliquely radial) should be smooth and clean, as a rough or dirty surface is liable to give a wrong impression of the amount of starch present. It must be borne in mind that, although one part of a specimen contains a certain amount of starch, the remainder will not necessarily contain starch to a similar degree.

It is a matter of great economic importance that *Lyctus* beetles will not normally lay more than an occasional egg in starch-free timber and that larvae hatching from such eggs cannot find nourishment and soon die, because removal of the starch affords a means of protecting susceptible timbers against *Lyctus* damage. The connection between starch content and borer attack (*Anobium*, *Lyctus*) in wood was first suggested by Mer (23, 24), who showed that timber could be rendered starch-free by certain methods of barking, girdling or topping the standing tree during spring and felling the following autumn. He also stated that starch could be caused to disappear from newly felled logs if they were left on the ground for a few months before conversion. The risk of fungal decay is considerable, however, with the last method, but he suggested that this difficulty could be overcome by storing the logs on skids under cover in well-ventilated premises. The disappearance of starch from newly felled timber has been confirmed by Wilson (35) and by the writer, who found that Mer's suggestion with regard to the method of storage renders the timber much less liable to fungal decay, especially if the ends of the logs are creosoted and tarred immediately after felling. Mer's results, however, need further investigation before they can be applied on a commercial scale. On the other hand, it may be possible, by some means other than those already mentioned, to remove starch or to alter it so that it becomes no longer suitable for assimilation by *Lyctus* larvae.

DISCUSSION

Wood may be considered to consist of skeletal substance and cell contents, and Campbell's (5, 6) comparative analyses of the wood and frass have proved quite definitely that *Lyctus* larvae are unable to alter any of the true skeletal constituents, but do cause a reduction in starch content. The work of Mer (23, 24) and Wilson (35) has recently been confirmed with reference to many Australian hardwoods by Cummins and Wilson (7), who have shown that, in a timber store generally infested with *Lyctus*, active attack was present only in those boards the sapwood of which contained more than a "trace" of starch. A similar result was obtained when numerous *Lyctus*-infested specimens of timber from the insectaries at Princes Risborough were examined for starch content.

As wood extracted with ether or alcohol is suitable for larval development, it is evident that *Lyctus* does not depend upon fats and oils in the wood for food. The substances present in the 60° C. water extract of oak sapwood have not yet been investigated, but it would seem from the results of the artificial feeding experiments that soluble sugars are likely to be the most important constituents. If it is only the lack of sugars, however, which retards the larval growth and causes such heavy mortality of young larvae, it is difficult to understand why the larvae should not develop more or less normally in water-extracted samples in which a plentiful supply of carbohydrate is present in the form of starch, since this starch must be hydrolysed to soluble sugars before the gut can absorb it. Some other essential factor, perhaps in the nature of a vitamin, may also be removed by the water.

In the past it has often been stated that one of the main problems connected with wood feeding is to account for the way in which the insects obtain a sufficient supply of nitrogen. Hawley and Wise (14) state that wood usually contains less than 0·3 per cent. of nitrogen. If we assume that 0·2 per cent. is a reasonable figure, this is equivalent to 1·25 per cent. of protein, so that the carbohydrate : protein ratio for wood is about 3·3 : 1. For comparison, the carbohydrate : (cell content) protein ratio for the normal 3000 calories basic diet for man is 4 : 1, so that *Lyctus* larvae might be expected to obtain their nitrogen supply without difficulty directly from the wood, a view which has also been advanced by Ripper (27) and Mansour and Mansour-Bek (22).

With reference to the presence of proteolytic ferments, it may be said that pepsin, although tested for, would hardly be expected to occur in the gut of a xylophagous insect, since Biedermann (1) has shown that the proteins of plant plasma are not digested by this ferment. Trypsin, on the other hand, actively attacks plant proteins, unless they are protected by alcohol-soluble lipoid substances. Although the photographic plate method gives no information upon the type of protein enzyme present in the larval gut of *Lyctus*, it would seem from the action of the ferment upon the gelatin, somewhat hardened by sodium metabisulphite in the fixing solution, that it has an action similar to trypsin.

Work on the subject of the food relations of the *Lyctus* powder-post beetles is being continued in an effort to establish a practical method or methods of rendering susceptible timbers immune from attack by reducing the nutritive value of the cell contents of the sapwood.

SUMMARY

1. The histology of the larval gut of *Lyctus* is described: owing to the absence of salivary glands and caeca, the digestive juices must be secreted by the midgut with doubtful contribution from the hindgut.
2. The mycetomes contain organisms which are apparently not symbiotic in relation to digestion.
3. The skeletal substance of wood passes through the larval gut unaltered, nourishment being derived from the cell contents.
4. A substance, soluble in water at 60° C., is necessary in oak sapwood for normal larval development.
5. Larvae are unable to develop in wood from which starch is absent.
6. Larvae living in wood containing relatively little starch may take two years to complete their development, instead of the normal one year.
7. Enzymes capable of hydrolysing starch, maltose, sucrose, lactose and protein were detected in tissue suspensions of the gut and its contents.
8. By feeding larvae on artificial diets it has been shown that starch, sugars and protein are necessary constituents of the larval food. Furthermore, larvae have been reared to the imaginal stage on a substrate which contains no wood.
9. The ability of the female beetles to determine the suitability of wood for larval development is demonstrated and the importance of the so-called "tasting" marks discussed.

10. An account is given of the occurrence of starch in timber and the difficulties involved in its estimation and removal from the wood.

ACKNOWLEDGMENTS

I wish to record my thanks to W. A. Robertson, Esq., Director, Forest Products Research, for permission to publish this paper, to W. G. Campbell, Esq., Officer in Charge, Wood Chemistry Section, for guidance on problems connected with the chemistry of wood and, in particular, to Dr R. C. Fisher, Officer in Charge, Entomology Section, who has supervised the investigation and given much useful advice and criticism during the preparation of this paper. Other acknowledgments are made in the text.

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EXPLANATION OF PLATE XVI

Fig. 1. Tasting marks made by female *Lyctus* beetles on surface of oak sapwood. (×3.)

Fig. 2. Obliquely radial surfaces of oak sapwood after application of iodine test to show the amount of starch present. *A*, abundant; *B*, moderate; *C*, little; *D*, none. (×16.)

Fig. 3. Starch-bearing and *Lyctus*-infested tissue occluded in oak heartwood. The transverse surface of the specimen has been treated with iodine solution and shows the starchy tissue as a black area (×$\frac{1}{3}$).

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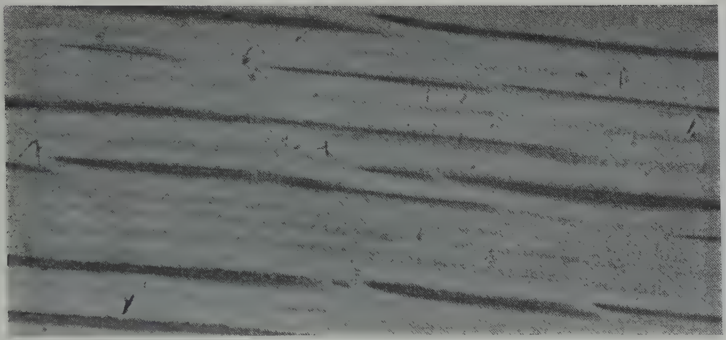
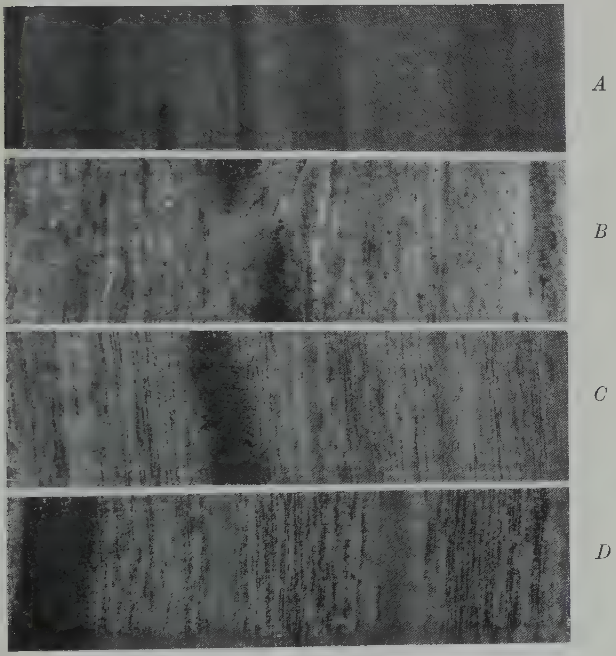


Fig. 1.



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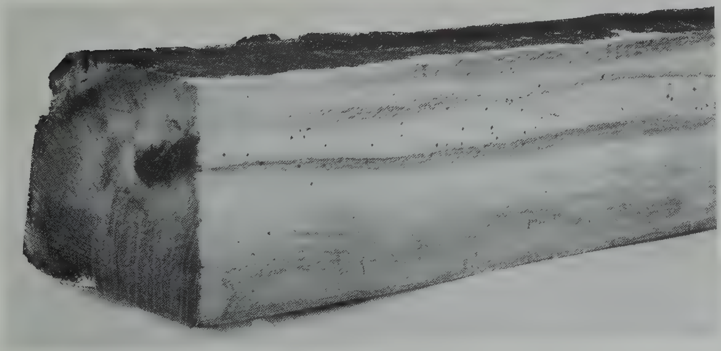

Fig. 3.

PARKIN.—A STUDY OF THE FOOD RELATIONS OF THE *LYCTUS* POWDER-POST BEETLES (pp. 369–400).

STUDIES ON THE APHIDES INFESTING THE POTATO CROP

V. LABORATORY EXPERIMENTS ON THE EFFECT OF WIND VELOCITY ON THE FLIGHT OF *MYZUS PERSICAE* SULZ.

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(With Plate XVII and 1 Text-figure)

Laboratory experiments have been arranged in order to ascertain, under controlled conditions, the reaction of winged aphides to the separate meteorological factors produced artificially. Such studies will form a basis for the consideration of data which are now being obtained *in the field* by means of a mechanical insect trap (Davies (5)) erected near the meteorological station at the College Farm, Aber. It will be possible by an examination of the aphides caught in this trap to correlate aphis migration with the several meteorological factors, either separately or conjointly. The effect of variation in relative humidity upon the flight of *Myzus persicae* has been studied in the laboratory and the results presented in a previous paper (Davies (4)). It was evident that high humidities definitely inhibit flight, and such evidence suggested an explanation of the low aphis infestation on potatoes in the humid districts of South Caernarvonshire and Anglesey compared with the heavy infestations in the drier areas of Flintshire (Davies (3)).

Generalisations are common concerning the influence of prevailing wind, or high winds, upon the migration of aphides and thus on the dissemination of virus diseases by these insects. It is desirable, therefore, to have precise data on the subject. The present paper deals with the results of experiments on the reaction of winged aphides to the wind factor produced artificially.

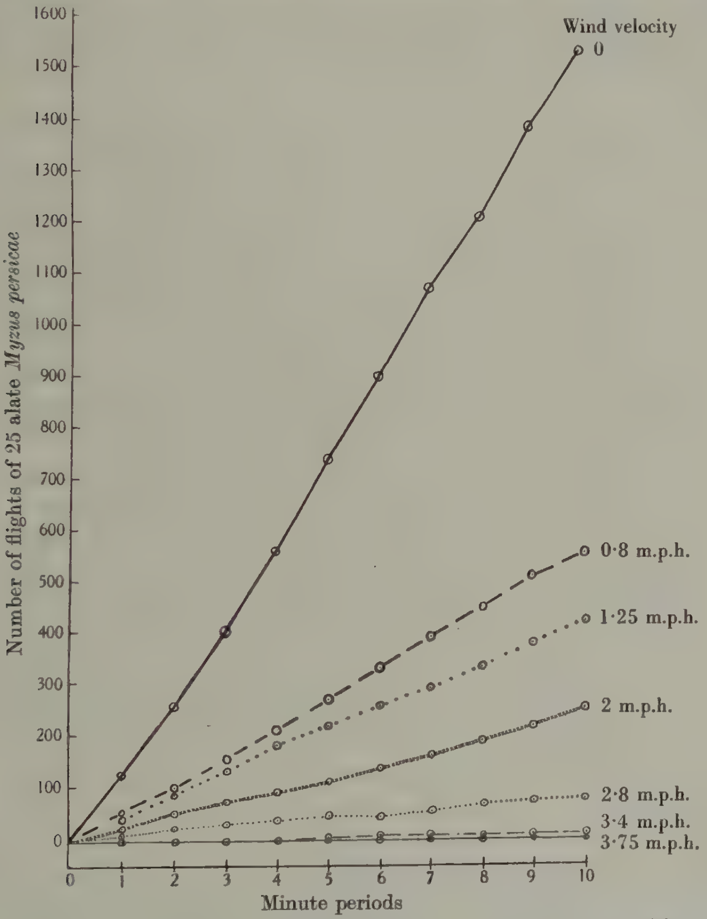
Technique

The apparatus used in these experiments can be described best by reference to Plate XVII, fig. 1. Wind was produced by means of a small electric motor as used in the familiar "hair-drying machine" (*A*). Variation in the velocity of the wind was obtained by means of a rheostat (*B*) and an extra resistance in the form of a 60-watt electric bulb (*C*). Some difficulty was experienced in accurately measuring the velocity of the wind. The Biram's anemometer (*E*) which is generally used for measuring wind velocity was not applicable for a jet of air less than the diameter of its fan. Since, however, the reading of the anemometer would be less than the actual speed, this instrument was used as a check and the figure recorded regarded as a mimimum. The only satisfactory method which could be used in the present case was the "smoke method" generally adopted for measuring the slow movement of air currents. It involved inserting a cloud of smoke (produced by wax tapers) at the mouth of the machine and allowing the smoke to pass along a length of glass tubing, equal in diameter (2 in.) to the mouth of the machine, and then, by means of a stop-watch, measuring the time taken for the smoke to travel a distance of 10 ft. along the tube. The wind velocity was checked at intervals throughout each series of experiments. As a further record of the speed of the wind a "flame anemometer" was placed at the exit of the experimental chamber (*G*) so that the angle at which the flame was blown gave a reading, previously calibrated, for the wind velocity. The experimental chamber (*G*) consisted of a glass cylinder (8 in. long, 2 in. diameter) attached at the end of the longer length of tubing by plasticine and, across the exit, coarse mosquito netting prevented the aphides from escaping.

Since these experiments aimed at ascertaining the effect of wind velocity when conditions were most favourable for flight, it was not necessary to control other factors, such as humidity, light and temperature, with any degree of accuracy. It was only necessary to carry out the experiments when conditions were most favourable and flight was more or less incessant. The dry air of the laboratory (below 45 per cent. relative humidity), a temperature above 70° F. and the light of a 500-watt lamp (*F*) were, in fact, ample to produce incessant flight. In all experiments, however, conditions were not regarded as optimum unless the 25-alate *Myzus persicae* placed in the experimental chamber gave a count of over 120 flights per minute when no wind was passing through the experimental chamber. These "controls" were used as a check, simply by switching off the machine after each variation in wind velocity, and the number of flights in the absence of wind was thus frequently recorded.

RESULTS

The results of the experiments are given in Text-fig. 1. The number of flights for each minute period is plotted on the curve for each wind velocity. A series of ten separate minute records constitutes the curve for each wind velocity. The results of the "controls" are given in the curve for 0 m.p.h. wind velocity. The very marked effect of wind upon flight is quite obvious in Text-fig. 1 Flights ceased when a wind velocity of 3·75 m.p.h. passed through the experimental chamber. Even at 2 m.p.h. the number of flights per minute dropped to an average of 25·2 as compared with the average of 154·8 flights per minute when there was no wind. While there were no flights when the speed of the wind was above 3·75 m.p.h. the aphides continued, intermittently, to crawl, mainly against the wind, but when the velocity was increased to 6·8 m.p.h. the aphides ceased crawling and remained more or less stationary. When the wind velocity was increased to gale force of 20–30 m.p.h. the aphides



Text-fig. 1. Rate of flights of *Myzus persicae* in varying wind velocities.

still remained on the smooth surface of the glass. It was surprising to find that even when the full force of the wind from the machine was passed through the experimental chamber the aphides remained on the glass with ease. The full force of the wind represented "hurricane" conditions, and the minimum record of the Biram's anemometer registered 70 m.p.h. When the wind was stopped by switching off the motor, the aphides immediately took to the wing.

METHOD OF ADHERENCE OF APHIDES DURING HIGH WIND VELOCITIES

It was of interest to ascertain precisely how the winged aphides were able to adhere to the smooth surface of glass during the winds of high velocity. Live specimens were, therefore, placed under a large cover-slip, which was slightly raised from the microscopical slide by means of wax feet. The wind from the electric motor was then directed on to the slide and the aphides were observed, upside-down, under the microscope. Plate XVII, fig. 2, is a photograph of an inverted live aphis adhering to the surface of the cover-slip in a wind of over 70 m.p.h. It was observed that there is a small membranous pad between the claws (*ungues*) and another at the base of each tarsus.[1] These pads are adpressed to the surface and each foot, alternately, is drawn very slowly towards the body. When a leg is completely withdrawn it is suddenly thrust out again and the pads adhere when the leg is fully extended; the process is then repeated. A photo-micrograph of the adhering tarsus of a winged specimen during the "hurricane" conditions is seen in Plate XVII, fig. 3. Since it is a time exposure the sharpness of the lateral outline indicates little side movement, whereas the fogged distal portion indicates the slow movement towards the body.

DISCUSSION

The present experiments have demonstrated the reluctance of winged *Myzus persicae* to take to the wing when the velocity of the wind exceeds 3–4 miles per hour. In other words, it is only when the wind velocity drops below this figure that the aphid expands its wings, rendering it buoyant, and prepares for flight. Only in such light breezes, therefore, can *voluntary migration* take place. So far as the problem of dissemination of virus diseases is concerned, it will be the prevalence of opportunities for this voluntary migration, when aphides can alight readily, take to the wing with ease and pass from plant to plant, that will be primarily involved.

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[1] Possibly this is the elastic membrane referred to by Theobald in *British Aphides* (1926), I, 13.

In winds of high velocity (20–30 m.p.h. and over), when the aphides adhere tenaciously to the surface on which they stand, there is no attempt to expand their wings. When, in the laboratory, they were pushed off with a needle in such high winds they were simply carried as inert bodies, tending to fall rather than rise. The incidence of such *involuntary transportation* in the field is being investigated.

In reviewing the literature on the subject of wind velocity and aphis migration it is surprising to find how vague and generalised are the statements concerning it. The term "wind" is applied equally to mere drifting air currents and to gales. In fact, no instance has been noted where the actual velocity of the wind has been measured during active aphis migration. The most recent work specifically designed to ascertain the relation between wind and aphis migration is that of Roney (11) in America, when he considered the effect of wind distribution of winged *Rhopalosiphum pseudobrassicae* Davis (turnip aphis) on the infestation of truck crops. Traps were erected close to plantings of truck crops and were placed in situations relative to the direction of the prevailing wind. It was found that the total catch (individual catches are not given) on the three traps placed on the lee side of the crops was almost six times as great as that on the three traps on the windward side. Whereas, at first, this would appear to prove a definite correlation between aphis migration and the direction of the prevailing wind such a generalisation does not necessarily follow. The "prevailing wind" will include winds of high velocities in which little or no aphis migration will take place so that it is only a fraction of the prevailing wind with which we are concerned. It does not follow invariably that the direction of this fraction, namely that of the light breezes, is always the same as that of the prevailing wind. In the experiments referred to there is no indication of the velocity of the wind concerned or the prevalence of winds favourable to aphis migration. In fact during three months of the year the catch, admittedly slight, on the traps on the lee side was actually less than on the windward side.

Statements concerning an association between aphis migration and the direction of the prevailing wind are commonly met with, but it would seem, in view of the present series of experiments, that it is more important to know the precise nature of the prevailing wind, since both its velocity and the degree of humidity will affect aphis migration. Cognisance, however, must be taken of the statements that aphis migration occurs when the wind is from a certain direction. For instance, Ainslee (1), when referring to the serious outbreak of *Toxoptera graminum* Rond. in 1926, states: "As was the case in Kansas in 1907 it seems that these aphides rose into the air in Minnesota only when the wind was from the South." Wadley (12) also supports the view of distribution of this grain aphis by southerly winds. Maxson (9), on the other hand, considers that in northern Colorado the beet aphis, *Pemphigus betae* Doane, is spread by westerly winds. The direction of the wind, in itself, would seem to be of little importance, but this correlation with direction, in different parts of the world, would suggest that such winds have certain factors in common. In North Wales, for instance, considerable migration of aphides has been observed when easterly and south-easterly winds are blowing. In this case, the east winds during the months of migration are seldom more than light breezes of less than five miles per hour, and are invariably drier than the prevailing winds from the opposite direction. The field experiments referred to are designed to obtain data on the precise nature of the weather conditions during maximum aphis migration.

It is generally held that "wind" is responsible for long-distance transportation of winged aphides. For instance, wind is claimed to be responsible for the presence of aphides on the islands of Memmert and Heligoland, some 15 and 39 miles, respectively, from the mainland (Borner (2)). Observations of Webster and Philips (13) indicate, also, that *Toxoptera graminum* Rond. covered over 100 miles through the agency of the wind. Wadley (12) similarly explains the sudden appearance of migrants of the same species in Minnesota, some 200 miles from the nearest source. But there is no data concerning the velocity of such winds. The only clear evidence of aphides actually observed in transit over long distances in "*strong* wind" is that of Elton (6), when he described swarms of aphides (*Delachnus piceae* (Walk)) being encountered on the ice-cap of North-East Land, Spitzbergen, suggesting transport over some 800 miles from the nearest host plant (*Picea obovata*) in the Kola Peninsula. Although, clearly, a case of abortive colonisation, it would be of interest to know what were the conditions when the aphides left their host plant. It seems unlikely that they would be blown off in swarms but rather that conditions on leaving their host plant favoured voluntary migration, and, subsequently, winds of high velocity transported them.

It would appear, as the result of the present experiments, that light air currents and drifts will play a far more important part in normal migration than the prevailing winds so often credited with the spread of aphides. Convection, and other upward air currents, the physical effects of streams, lakes and mountains upon these air drifts, as suggested by Felt (7), will need to be considered in the question of aphis migration from crop to crop.

SUMMARY

The results are given of laboratory experiments on the effect of variation in wind velocity upon the flight of *Myzus persicae* Sulz.

When no wind passed through the experimental chamber, twenty-five winged aphides averaged 154·8 flights per minute. The incidence of flight in a range of wind velocities is recorded. Low wind velocities had a marked influence on flight which ceased when the speed of the wind was increased to 3·75 m.p.h.

The aphides remained stationary on the glass surface of the chamber when high winds of 20 and 30 m.p.h. passed through the chamber and even when the full force of the wind, equivalent to a gale of 70 m.p.h., passed over them the aphides adhered to the surface with ease. Microscopical observation showed that the aphides possess a small membranous pad between each claw and another at the base of each tarsus; these are adpressed to the surface and facilitate adherence.

The literature on the subject of the dissemination of aphides by wind is reviewed and the phenomenon of *voluntary migration* as compared with *involuntary transportation* is discussed.

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EXPLANATION OF PLATE XVII

Fig. 1. *Experimental apparatus.* *A*, electric "hair-drying machine" used to produce wind; *B*, rheostat inserted to produce variation in wind velocity; *C*, electric bulb (60 watt) as extra resistance; *D*, ammeter, to control current; *E*, Biram's anemometer; *F*, 500-watt lamp for lighting; *G*, experimental chamber; *H*, whirling hygrometer.

Fig. 2. Winged *Myzus persicae* adhering (upside-down) to surface of cover-slip during wind velocity of over 70 m.p.h. × 30.

Note. Adpressed tarsi.

Fig. 3. Tarsus of winged *Myzus persicae* closely adpressed to cover-slip while adhering (upside-down) in wind of over 70 m.p.h.

Note. Sharpness of lateral outline indicating little side movement. Also fogged distal portion indicating slow movement towards the body. × 370.

(*Received October* 28, 1935)

PLATE XVII

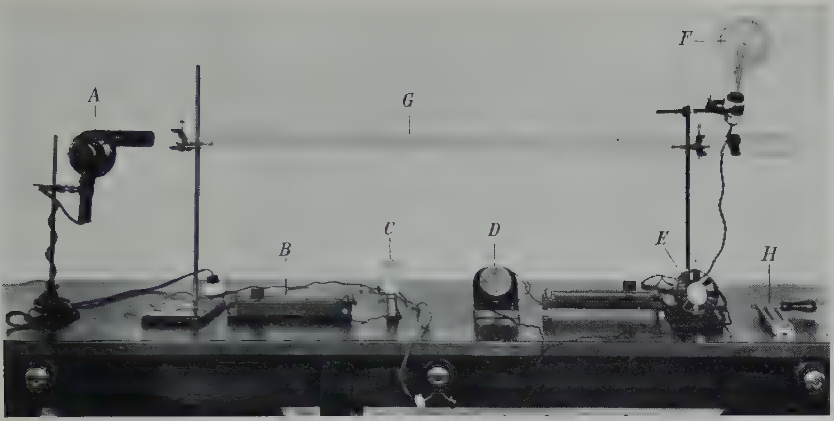

Fig. 1.

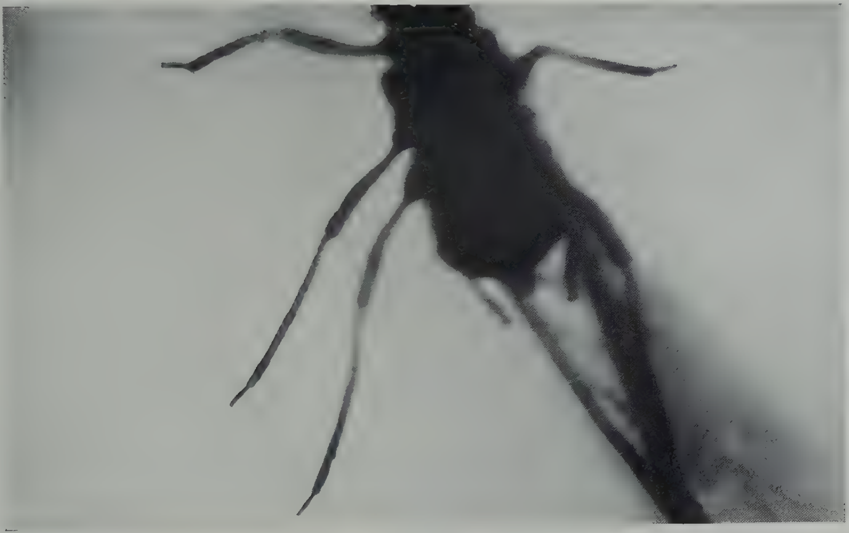

Fig. 2.

Fig. 3.

DAVIES.—Studies on the Aphides Infesting the Potato Crop (pp. 401–408).

SOME NOTES ON THE BIOLOGY AND ECONOMICS OF SOME BRITISH CHAFERS

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(With 2 Text-figures)

Introduction

The adult beetles of the subfamily Melolonthinae are usually known as chafers, maybugs or June beetles, while their larvae which are all soil inhabitants, are often referred to as white grubs or rook worms. The subfamily contains some of the most important and well-known species of the Scarabaeidae, a family of beetles which has since ancient times been recognised as of great economic importance. Little work has, however, been done in this country on the biology of the Melolonthinae, although some papers have been published on the control measures adopted against them. On the Continent the biology of *Melolontha* sp. has been studied extensively by Janke (4) and other authors, but, on the other hand, little seems to be known of the habits of *Amphimallus*, and the writer has been unable to find any account of the biology of *Serica*; in the British Isles, these two latter species are almost as important as *Melolontha*.

A study, extending over two years, of the life history, habits and economics of *Amphimallus solstitialis* L., the summer chafer, and *Serica brunnea* L., the brown chafer, was therefore undertaken with a view to comparison with the accounts of *Melolontha melolontha* L., the common cockchafer, as given on the Continent and in this country. Some notes on the other species of the subfamily are also appended.

A. Economics

(1) *Crops attacked*

The damage done to crops by chafer larvae is almost entirely restricted to those parts of the plant which are below the soil surface. *Serica* has, however, been known to attack the stems of young plants just above this level, and the writer has noted, when feeding very young (first year) larvae of *Amphimallus* on cabbage seedlings about 1 in. high, that the larvae did not feed on the roots but emerged from the soil and attacked the stems. These stems were usually ringed just above the soil, and in collapsing gave an appearance very similar to "damping off" of seedlings. This habit of girdling stems or roots, especially of young trees, is said to be typical of *Amphimallus* and *Serica*, whereas *Melolontha* generally bites right through the plant.

The more usual damage to plants is the removal of the side roots, and in badly infested areas where there is only a sparse vegetation *Melolontha* will destroy all the roots. The writer has observed wilted plants which were found to have only a short stem embedded in the soil, entirely lacking in roots, and near which one or more chafer larvae were discovered in the soil. In grassland these larvae usually eat all the roots at a given level from 2 to 4 in. below the surface. In severe infestations, the grass dies and can be rolled off like turf; early stages of such an attack may be recognised by the sponginess of the turf underfoot.

Records of crops damaged by chafer larvae are given in Table I. They are taken mostly from the *Monthly Summaries of Plant Pests and Diseases in England and Wales*, issued by the Ministry of Agriculture and Fisheries; but other reports of damage done by chafers in Britain, which have come into the writer's hands, have also been included. The figures show the number of records during the years 1914–34.

Table I

Crops attacked by chafer larvae

Crop	*Melolontha*	*Amphimallus*	*Serica*
Brassicae	7	1	—
Cereals	5	—	—
Clover	—	1	—
Garden crops	7	2	—
Grassland	10	7	4
Mangels, turnips	2	1	—
Orchards	3	1	—
Potatoes	12	1	1
Pyrethrum	1	3	1
Roses	4	—	—
Strawberries	18	—	—
Sugar beet	4	—	1
Tree seedlings	4	2	8
Miscellaneous	3	4	4
Total	80	23	19

It will be seen that the total number of reports of *Melolontha* far exceeds those of either of the other two species. Reports of the occurrence of *Serica* were confined to the last 5 or 6 years only, while those of *Amphimallus* go back about 15 or 20 years. Records of *Melolontha* doing damage, on the other hand, cover a considerable period, both Curtis (1) and Ormerod (8) recording it as a pest between 50 and 100 years ago. The lack of records of *Serica* and *Amphimallus* is unlikely to be due to these species having become pests only during the last few years, but is probably accounted for by their larvae having been confused with the young stages of *Melolontha* and the damage thus assigned to this species.

It will be noticed from Table I how extremely varied are the types of plants devoured, there being in fact very few crops which the grubs are known to avoid. Zimmermann (11) records that poppies, although consumed by the larvae if there is not alternative food, are poisonous to them; and the writer has found that Sweet William (*Dianthus*) is not usually damaged even in soil heavily infested with *Melolontha*. Similarly mustard does not seem to be very favoured by chafers. The larvae of *Melolontha* are commonly reported infesting sugar-beet crops on the Continent, and it is of interest that this has not often been noted in this country. Various authors state that in their early stages chafer larvae feed almost entirely on humus. The writer has, however, been unable to find any evidence for this, and, on the contrary, these stages have been observed to feed readily in captivity on young cabbage and grass seedlings.

The damage caused by swarms of adult beetles eating the leaves and flowers of crops is not large in this country. On the Continent severe damage is caused to woodland plants. In the British Isles, although some losses on this type of crop have been recorded, the beetles are more injurious to fruit trees, but they do not seem to attack pears and usually avoid lime and Robinia. On the Continent *Melolontha* is said to have preferences for oak, but occurs on chestnut, cherry, plum, maple, larch, withy, alder and apple. *M. melolontha* prefers alder and walnut and *M. hippocastani* mountain ash. It will thus be seen that the preferences on the whole are for fresh juicy leaves as opposed to the harder varieties.

(2) *Distribution*

The occurrence of chafers throughout the British Isles is determined by two main groups of factors. The first of these groups comprises the physical and chemical nature of the soil and the type of ground vegetation and is considered here under the heading of "Habitat". If there is a suitable habitat present in a given area, it is also necessary that the second group of factors making up the external environment, chiefly the prevailing meteorological conditions, should be favourable; these will be discussed under the heading of "Locality".

Habitat. It is extremely difficult to define in any detail the nature of the habitat in which chafers usually occur, but there seem to be certain preferences for each species. As far as soil conditions go, there is a general preference shown by all chafer species for light, dry and sandy soils. This is possibly due to the fact that high percentages of humidity tend to favour the development of fungoid and bacterial diseases; and since the larvae are soft and fleshy, they may encounter some difficulty in moving through heavier soils. This last theory is supported by the fact that *Melolontha* larvae, which are the largest and strongest of the three genera under consideration, are often found in soils which are far heavier than those preferred by the others. *M. hippocastani* is nevertheless said to favour a soil rather sandier than that in which *M. melolontha* usually occurs. Normally light-coloured soils are more favoured than darker forms, and where both occur together much lower numbers of larvae will be found in the latter.

As to ground vegetation, the species seem to differ widely in their preferences. The factors which control these are apparently the proximity of adult and larval food plants, and the presence of a ground vegetation which will give suitable conditions of temperature and humidity in the soil, and also be favourable for oviposition. Both species of *Melolontha* prefer ground near such trees as oak and pine, but *M. melolontha* usually occupies more open ground covered with rough grass and a few scattered trees or in the proximity of a wood. *Serica* is also usually found under trees, especially oaks, with a ground vegetation of mossy grass and bracken; it does, however, sometimes occur away from trees if such a ground vegetation is found near hedges. Both species of *Amphimallus* occur in soil unshaded by trees but covered with grass or rough vegetation.

Locality. On studying a large area where climatic conditions are favourable to chafers, it is found that these beetles do not occur uniformly throughout, but at certain scattered points. The distribution is in fact what is known as local. This localisation is caused by two factors: firstly, the comparative scarcity of suitable habitats as described above; and secondly, the fact that adult chafers do not migrate from one area to another to more than a slight extent. The females of *Melolontha* very seldom fly more than half to three-quarters of a mile from the larval habitat from which they emerged, and it is a characteristic of chafers as a whole to oviposit in the field in which they themselves developed. Therefore, even if neighbouring fields form perfectly suitable habitats, they may not be infested, or if they are, they may be occupied by a totally different flight of beetles. This is of importance in accounting for flight-years, as will be discussed later.

The two main factors which appear to determine the distribution of chafers are temperature and humidity; barometric pressure is not known to have any direct effect. Since these factors are of much more importance during the relatively long and sensitive larval period, it is soil temperatures and humidity which should be studied rather than air temperatures and rainfall. It is unfortunate that figures for soil humidity and to a lesser extent soil temperatures are not available, owing to the lack of suitable recording instruments. Figures for air temperatures are, however, at hand for many years, and they have a marked bearing on the distribution of chafers.

It is a common practice to compare distribution with the mean annual isotherms, but since chafer larvae hibernate in a comatose and insensitive state at a fairly low level in the soil, it is probable that the winter temperatures have little effect on them. Greater accuracy may therefore be obtained by using isotherms for the summer months between April and October, of which the mean July isotherms shown in Fig. 1 give a fairly representative idea. It will be noticed from Map I, which compares the distribution of *M. melolontha* with that of *M. hippocastani*, that the former occurs as a pest where the mean July temperature is above 15·5° C., but it has also been reported from localities where this figure is as low as 14·5° C. *M. hippocastani*, on the other hand, occupies localities in which the summer temperature is less than 15·5 but greater than 14·0° C. Figures are also available for the distribution of these two species on the Continent. Zweigelt (12) states that *Melolontha* sp. do not occur as pests in localities where the mean annual temperature is less than 12·5° C. Both species are pests in Germany, France, Switzerland, Austria, Hungary, Jugo-Slavia, Holland, Denmark and Russia, west of a line drawn from the Baltic to the Caucasian Provinces. The extreme limits are thus between the 12·5 and the 22° mean summer isotherms.

In Map II the distributions of *Amphimallus solstitialis* and *A. ochraceus* are compared with the mean July isotherms. It will be seen that the former species occurs as a pest in localities where this temperature is above 16·5° C. and occasionally where the temperature is below this but above 15·5°. *A. ochraceus*, which is a much rarer species, does not seem to occur in areas with a temperature below 15·0 or above 16·5° C., except in one instance, namely, that reported by Joy (5) in 1904 at Streatley, Berks. Neither species seem to be known in Ireland. On the Continent, the distributions of these two species differ widely. While *A. ochraceus* is very local and occurs down the coast of France and Spain and on the north coast of the Mediterranean, *A. solstitialis* does not seem to be known farther south than Switzerland, although it is plentiful in Russia and as far north as Sweden.

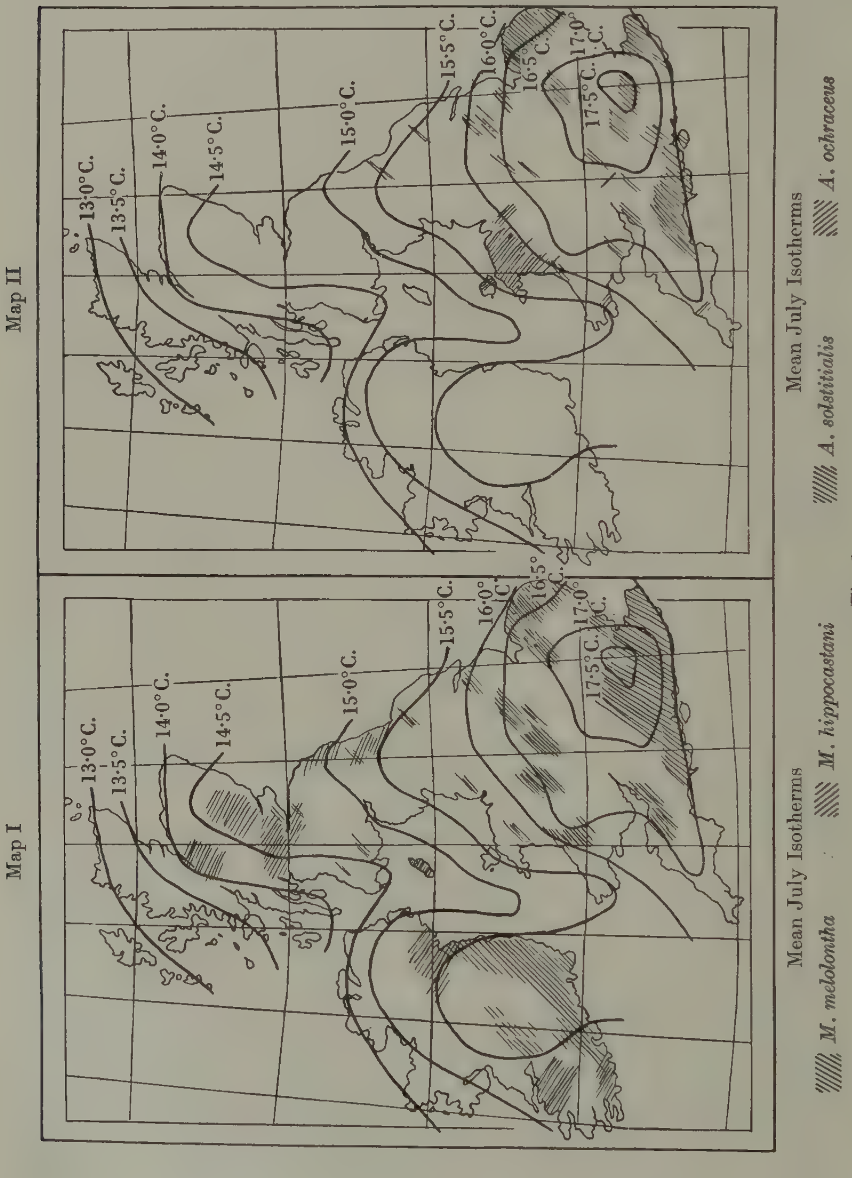
Map I
Map II
13·0°C.
13·5°C.
14·0°C.
14·5°C.
15·0°C.
15·5°C.
16·0°C.
16·5°C.
17·0°C.
17·5°C.
Mean July Isotherms
M. melolontha
M. hippocastani
Mean July Isotherms
A. solstitialis
A. ochraceus

Fig. 1.

The reports of *Serica* are unfortunately a great deal fewer and consequently harder to classify. This species seems, however, to occur locally but fairly plentifully throughout England, except perhaps in the south-east; it has been reported several times as a pest in the Lowlands of Scotland and is a common pest in Ireland. On the Continent, it occurs more to the north and is plentiful in Sweden.

Very much less is known about the effects of humidity on the distribution of chafers. This is partly due to the fact that soil humidity does not bear such a direct relationship to rainfall as soil temperatures do to air temperatures, being controlled by the type of soil and the ground vegetation present. Thus any small-scale isometric map showing soil humidities would be extremely difficult to plot and very complicated in appearance. It is, however, possible that the uneven distribution within areas of equal summer temperatures may be partly caused by local differences in rainfall. Decoppet (2) has shown by observations over a long period in Switzerland that rainfall can have a marked effect on the periodicity of flight-years, and it thus seems that it probably affects the life history and distribution.

Altitude has its effect on the distribution, probably connected with the fall in temperature with increase in height, rather than with the fall in barometric pressure. This is supported by the evidence that the respective heights at which *Melolontha* sp. cease to occur differ widely in different areas; thus in the Southern Alps the limit is 1300 metres, in the Bavarian Alps 600 metres, while in Scotland *M. hippocastani* does not appear to occur much above 450 metres.

B. BIOLOGY

As the larvae of chafer beetles are soil inhabitants, it is almost impossible to make close and continuous observations on them in their natural habitat. Moreover, the grubs are very liable to develop fungoid and bacterial diseases in captivity, especially in the autumn months, and they cannot be handled, since the least touch develops into a bruise and fatal mould; also barely 20 min. of sunlight causes death. A detailed knowledge of the immature life history is thus very difficult to obtain.

During the present studies, the larvae were placed in 2–3 oz. glass-topped tin boxes, filled with fine sandy soil. Since an earthen dug-out, as recommended by several American writers, was not available, these boxes were kept in a cellar facing north and east, which had a constant temperature of 12·0–14·5° C. and relative humidity of 78–80 per cent. in both summer and winter. These were not, however, found to be ideal conditions, since they induced the larvae to commence hibernation rather early and to continue rather late, thus causing a lengthening of the larval stage. The larvae were fed on seedlings of cabbage, grass and wheat. The results of these studies are summarised in Fig. 2.

(1) *Egg stage*

The eggs, which have been described elsewhere (3) by the writer, are usually laid in rather damp light soil, at a depth of about 4–5 in. in the case of *Melolontha*, but at rather less than this with the other two species. A higher soil humidity is needed for the development of the eggs than is favoured by the older larvae which obtain some moisture from their food. Thus a sudden drought during the egg stage often proves fatal. The appearance of the eggs alters slightly during development; they lose their typical round whiteness and become rather elongated, one end being creamish. Just previous to hatching, one side of the egg becomes somewhat translucent and the embryo larva can be seen through the chorion, curled up on the other side.

The length of the egg stage seems to be even more variable than that of the larvae. With *Melolontha* sp. on the Continent, the eggs are said to take 468 hours to develop. The writer has found, however, that at least under artificial conditions, the eggs of *M. melolontha* require up to 28 days, and different individuals of a batch laid simultaneously by a single female may vary a great deal, a week transpiring between the time of hatching of the first and last eggs. The egg stage of *Amphimallus* is usually a little shorter; the time occupied in development may be only 18 days, though in artificial conditions it may be as much as 28 days. With *Serica* the egg stage covers an even shorter period, as is to be expected from its being a considerably smaller size. The first eggs begin to hatch after 15 days and all hatching is completed by the end of 22 days.

Table II

Length of egg stage (in days)

	Maximum	Minimum	Mean
Melolontha melolontha	28	20	25
Amphimallus solstitialis	28	18	23
Serica brunnea	22	15	17

Although the length of the egg stage is probably affected by temperature and humidity, there must be some other factor, possibly hereditary, to account for the differences in the individuals of a batch exposed to identical climatic conditions. It is thus only possible to estimate roughly

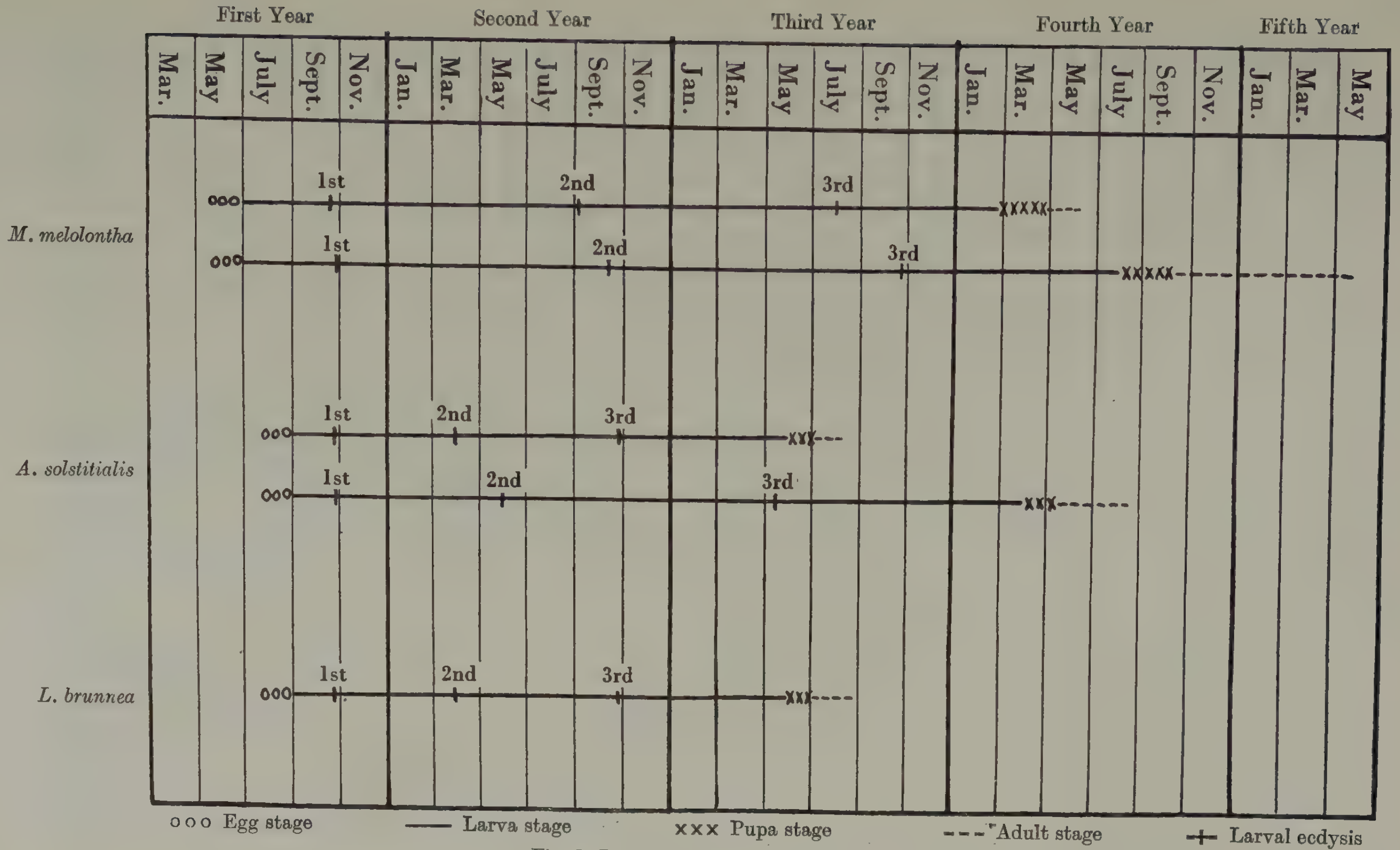

Fig. 2. Life cycles of the Melolonthinae.

the period an egg will take to develop and to forecast the average time of hatching of a batch.

There is, as stated elsewhere, no special "egg burster" on the young larvae of this subfamily, and hatching is apparently brought about by the embryo larva biting through the chorion with its mandibles. On hatching the larva is completely white and soft except for the tips of the mandibles, the usual colouring being assumed after a few hours. The chorion is usually, but not always, consumed, and the young larva is capable of living from 2 to 3 days without further nourishment.

(2) *Larval stage*

Length of development. In Continental countries, the length of the life history is more or less fixed according to the district. This period for *Melolontha melolontha* may be either 3, 4 or 5 years, but is always regular in any given district. Zweigelt (12) maintains that these various times are due to differences in local temperatures, while Decoppet (2) and Schmidt (10) hold that the length of the life history is fixed for all time by inheritance, there being several biological races each taking different times to develop; Decoppet admits, however, that rainfall may have some effect.

It has nevertheless been shown that *M. melolontha* can be bred out in as short a period as 18 months, and therefore the length of the life history must be variable. Any statement as to the length of the larval stage is useless unless some indication is given of the climatic conditions controlling it, and a study of the life history under artificial conditions will give little indication of the length of development in Nature, unless the local climatic conditions are also known; this subject is being treated more fully in a separate paper by the writer. It is, however, possible to obtain a fairly reliable estimate of the life history under natural conditions by comparing large numbers of specimens taken in the field with those stages which have been bred in the laboratory and studied under known conditions. Some results of such comparisons may be seen in Fig. 1. In this diagram, the alternative and longer times taken by *Melolontha melolontha* and *Amphimallus solstitialis* were recorded in artificial conditions, namely, a temperature of 12·0–14·5° C. and humidity of 78–80 per cent.; these probably represent more northerly climates than the natural conditions in which the shorter periods were observed, namely, in southern England.

While the larval stages of *Serica* last for about 21 months, those of *Melolontha melolontha* vary between 32 and 37, and of *Amphimallus solstitialis* between 21 and 31 months. Variations beyond these limits are not common, this being apparently due to the occurrence of hibernation between October and March, and of a semi-dormant stage, which lasts up to 2 months, before pupation. During this latter period, the larvae gradually alter shape, becoming rather broader in the middle of the body and pointed at the caudal end, thus assuming the shape of the pupa which is forming beneath. This change does not take place during the time of hibernation, when presumably the temperature is too low to allow of active metabolism, and, if the larvae are not sufficiently developed in the autumn to start pupation at once in the spring, feeding is necessary before this 2-month period can be commenced. With *A. solstitialis*, which does not pupate until rather later in the summer, the hibernation period is reached if pupation is delayed appreciably, and this stage has to be postponed until the following spring.

Continental writers state that the larvae of *Melolontha hippocastani* require a year longer to develop than those of *M. melolontha*. Thus in districts where the latter species takes 3 years to develop, *M. hippocastani* takes 4 years, and 5 years where *M. melolontha* takes 4 years.

A seasonal variation in relation to the soil temperature in the depth to which these larvae penetrate in the soil has been indicated in the works of McCollock and Hayes (7) and Mail (6), and is being dealt with by the writer in a separate paper.

Larval ecdysis. The method used for determining the date and number of larval ecdyses was an application of Dyar's law, since the discovery of the cast skins was considered too unreliable. The widths of the head capsules of fixed larvae were therefore measured, and it was found that there was a mean width for each instar of each species, and that although the individual measurements varied slightly about this mean, as illustrated in Table II, there was no overlapping. There appeared to be no indication of two groups formed by male and female larvae.

Table III

Width of head capsules of Serica brunnea (*in mm.*)

	Mean	Maximum	Minimum
First instar	1·23	—	—
Second instar	1·79	1·92	1·67
Third instar	2·35	2·38	2·31
Fourth instar	2·68	2·92	2·46

The method can be taken as giving fairly accurate results, and on applying it to the three species it was found that there were only three larval ecdyses in each, *i.e.* four larval instars. The mean widths of the head capsules are given in Table IV.

Table IV

Mean width of larval head capsules (in mm.)

	Melolontha melolontha	*Amphimallus solstitialis*	*Serica brunnea*
First instar	2·1	1·82	1·23
Second instar	3·0	2·44	1·79
Third instar	4·5	3·25	2·35
Fourth instar	7·1	4·40	2·68

Since this method of ascertaining the instar of a larva is only applicable to dead larvae, a series of measurements for any one specimen could not be obtained. The dates of ecdysis were therefore determined by fixing a number of specimens at frequent intervals and measuring their head capsules. This gave a very fair estimate of the mean time of ecdysis and was equally useful for both laboratory-bred specimens and those taken in the field, so that the periodicity of ecdysis in natural conditions could be obtained.

Since the total length of the larval life history varies as a whole, it is to be expected that the length of the individual instars will also vary. Fig. 2 illustrates that with all three species the first ecdysis usually takes place just before hibernation starts in the first year, *i.e.* the year of hatching; it may, however, in rare cases be postponed until the spring of the following year. With *Melolontha melolontha*, depending upon whether the individual larva is destined to take 3 or 4 years over development, the second and third ecdyses take place in the late summer or autumn of the second and third years respectively. With *Amphimallus solstitialis*, on the other hand, the second and third ecdyses may both occur in the second year, but if the larva is due to take 3 years over development, the third ecdysis is usually postponed until the spring of the third year. Similarly with *Serica brunnea*, although it has never been known during the present study to take 3 years to develop, the third ecdysis is sometimes postponed until the spring of the third year, *i.e.* until just before pupation.

(3) *Pupal stage*

As mentioned above, there is a distinct prepupal stage in the life history of chafer beetles. This is marked by the larva ceasing to feed and burrowing down into the soil to a depth which is fixed mainly by the type of soil and the prevailing conditions of temperature and humidity. The usual depth is about 6–8 in. for *Serica* and *Amphimallus*, and about 12–14 in. for *Melolontha*. This last species has, however, been recorded as pupating at a depth of 3 ft. or more.

The larvae form cells in the soil by pressing out the soil surrounding them into relatively hard packed walls. The hollow inside is more or less egg-shaped, and the larva rests in it curled up with its head uppermost. The prepupal stage usually lasts about 6 weeks with *Melolontha*, 4 with *Amphimallus* and 3 with *Serica*, and as it proceeds, the larva takes on a shorter appearance, becoming broader in the middle of the body and pointed at the caudal end. At the termination of this period the larval skin splits down the dorsal side; the pupa may wriggle out or if undisturbed remain in the old larval skin. The only movement of which the pupa seems capable is a flexing and unflexing of the abdomen which takes place if it is interfered with.

The length of the pupal stage does not vary quite as much as do the other stages of the life history, and in no instance in the writer's knowledge have any of the species under review hibernated as pupae. Fig. 2 shows that the larvae of *Melolontha melolontha* may pupate either in the spring or autumn of the fourth year. Those which pupate about March emerge as adults in May, but, if pupation is delayed until the following August, the adults which are formed after about 6–8 weeks' pupation remain in their cells until the coming spring, thus making a 4-year cycle. With *Amphimallus*, on the other hand, as has been explained previously, hibernation commences if pupation is delayed appreciably after June. Thus in the longer life cycle they hibernate as larvae and pupation, which lasts for 5 or 6 weeks, takes place early the following spring, the adults remaining in the soil until the usual time of emergence in July. With *Serica*, the time of pupation seems fairly fixed, starting early in June and lasting about 4 weeks.

(4) *Adult stage*

Hibernation and flight period. As stated above, it is usual for the adults of most chafer species to cast the pupal skin at least some days and at the most the previous autumn before they emerge. The beetles are at first soft and colourless, and they remain in the pupal cell for a varying time, gradually assuming a darker colour and becoming harder. As the weather becomes warmer and the date approaches at which the flight begins, the beetles dig their way towards the surface and then remain just below waiting for a suitably warm day to emerge. With *Melolontha*, the temperature has to be about 20° C.; with the other two species it is higher, since they appear later in the summer. Decoppet (2) states that the emergence of *Melolontha* is controlled by the sum of mean day temperatures after March 1, being about 355° C. In the south of England, according to the observations of the writer, *M. melolontha* usually appears at the end of the first week in May, *i.e.* about May 7–10. *Amphimallus solstitialis* emerges at the beginning of July, and *Serica* about the 5th to 10th of that month. *Melolontha hippocastani* is said to appear about 10–14 days before *M. melolontha*. All these dates of emergence are nevertheless often as much as 7–10 days earlier if the weather conditions have been warm and dry.

The adults of *Amphimallus solstitialis* take to wing at about 8.30 p.m. and continue until dusk at 10 p.m. *Melolontha* and *Serica* fly rather later, since swarming starts at sunset and continues until it is quite dark. It is probably for this reason that *Amphimallus* is less noticeably attracted to light than are the other two species which are often found swarming round lamp-posts or flying into lighted rooms. In the writer's experience, far more males than females of *Melolontha* are attracted to light. *Hoplia* and *Amphimallus ochraceus* differ from the rest of the Melolonthinae in that they fly in bright sunlight.

After the first week of the flight, the females collect in large numbers on favourite twigs and bushes, night after night, the males meanwhile flying round and round. The flight does not, however, extend over a distance greater than half to three-quarters of a mile, the beetles remaining in the same area year after year. Although swarms do not often attain large sizes in this country, they are very common on the Continent and are stated to be very difficult to stop or alter in direction once they have started. If the beetles come into collision with anything, the elytra are closed and they fall to the ground. Flights do not occur on cold or windy evenings, and a period of sharp frost may drive the beetles back into the earth or kill large numbers of them.

The beetles feed during the earlier part of the evening, swarming over the branches of trees and bushes and consuming the leaves and blossom. Large swarms may completely defoliate a fruit tree. During the day and the latter part of the night, the beetles bury themselves in the soil or undergrowth or, as is the case with *Melolontha*, they cling to the high branches of the tree tops. From frequent burrowing in the soil, the pubescence on the back of the two larger species, especially with the females, soon becomes rubbed off.

The sexes are usually stated to occur in equal numbers, at least at the beginning of the flight period, though later the females burrow into the soil to oviposit. In the writer's experience, however, males definitely predominate, especially in the case of insects bred out in captivity. Decoppet (2) states that as the flight period continues, the average size of

Melolontha adults gradually decreases, and he found that there were more individuals to a given volume of beetles collected. This he considers may be due to a predominance of males which are of very slightly smaller size. It is, however, possible that the adults which have been less well nourished in the larval state and are consequently smaller, emerge later.

Pairing and oviposition. Pairing takes place from 3 to 7 days after the beetles have emerged from the soil, the males seeking out the females which are resting on branches in the tree tops and bushes, towards the end of the evening flight. Pairing usually lasts for about 3 hours, but the males may remain in position for several days if the weather is unfit for a further flight. Each female usually copulates two or three times, but Decoppet states that *Melolontha* may pair up to five times.

According to Scheidter (9) there are usually five immature eggs in each of the six egg tubes of the ovaries of females of *M. melolontha* at the time of emergence. These eggs are slow to mature and about 2–3 weeks elapses before they are laid. The act of oviposition extends over 2 days, twenty to thirty eggs being laid. With this species, a second series is usually laid, and the above writer states that these eggs are more quickly matured than the previous batch, and that, although the same number are laid as in the first series, there are often three to five eggs left in the ovaries unlaid. The writer has found that for the most part *Amphimallus* and *Serica* lay only one series of eggs, these being about twenty-five to thirty-five in number with the former species and twelve to twenty-five with the latter. They are laid 10–14 days after pairing, *i.e.* about three weeks after the beetles have emerged.

For egg-laying, the females choose rather dry soil in a sunny situation and covered with rough vegetation such as grass. They dig down into the soil and lay their eggs 3–5 in. below the surface in the case of the two smaller species, but rather deeper than this with *Melolontha*. The eggs are usually scattered singly or in groups of two or three, though all of one series are within a foot of each other unless the female is disturbed in the act of oviposition. *Serica* differs from the other species in laying its eggs together in small compact balls.

Flight-years. It is well known that the adults of most chafers, and in particular *Melolontha* sp., occur in much larger numbers in certain years, which recur with fixed regularity, than in the years between. The length of the cycle coincides with that of the developmental period for the locality in question, and in the year before (*Vorflugjahr*) and the year after (*Nachflugjahr*), there is a slight increase above the intervening years, though the number are less than in the flight-year. This phenomenon is very noticeable and occurs with great regularity in most European countries, but is much less well marked in the British Isles. In Germany, Russia and Switzerland these flight-years have been studied over long periods by a number of workers, but some difficulty has been found in explaining them. The fact that chafers take several years to develop does not in itself account for this cycle, since, as with other insects such as wireworms, we might expect to find three (or four according to the life cycle) broods of larvae in the soil together and adults appearing in large numbers every year, or at least irregularly according to the climatic conditions.

The usual theory brought forward to account for this regularity is that the larvae of one brood devour any other younger larvae of successive broods which may be associated with them. Thus the hatching larvae are always in danger of extermination by older larvae; but when eggs are laid in large numbers, as they would be in the flight-year, there is much more chance of that brood surviving. This theory is supported by the evidence that the years of the flight-year may differ even in localities which are quite close together if the larvae are not in association with one another. There does not seem to be direct evidence that chafer larvae are cannibalistic, but the writer has noticed that with most species, and especially with *Serica*, they are very liable to attack one another, and if one member of a batch dies, its body, except for the hard head capsule, immediately disappears.

This explanation is incomplete however, in that it does not account for the slight increase in numbers of the *Vorfluge* and *Nachfluge*. Two main theories have been brought forward to cover this, namely, those of Decoppet (2) and Schmidt (10) on the one hand, and Zweigelt (12) on the other. The two former writers, on the ground of several years' observation in Europe, maintain that the larvae are of different biological races of fixed developmental time, taking either 3, 4 or 5 years, and are unaffected by variations in temperature, although Decoppet admits that rainfall may have some affect. He says: "Il établait (Raspail, 1891) au contraire que la génération de quatre ans a coincidé avec trois étés (1886–1888) particulièrement secs, et la génération de trois ans qui suivent, avec deux années humides."

Thus Decoppet and Schmidt assume that the beetles of the flight-year are of a different biological race from those of the *Vorfluge* and *Nachfluge*, whose larvae take shorter and longer times respectively to develop. There is, however, absolutely no evidence to show that these biological races really exist, and, on the contrary, it has been shown, as mentioned in the section dealing with larval development, that this period is definitely variable.

Zweigelt, on the other hand, maintains that temperature is the controlling factor. He points out in support of this, that in districts where the mean annual temperature is 7° C., flight-years of *Melolontha melolontha* occur every 4 years, while where it is 9° C. they are 3 years apart. If this temperature is 8° C., the larvae may take either 3 or 4 years to develop. Thus the *Vorfluge* and *Nachfluge* are caused by some of the larvae of the same brood as those of the flight-year coming under specially favourable or unfavourable conditions of temperature. In the opinion of most recent writers, this theory only falls short in that it does not account for the influence of humidity which probably has an effect similar to that of temperature.

The irregularity in the occurrence of flight-years of *Melolontha* in the British Isles would thus appear to be due to the annual variations in the British climate. In support of this it is found on the Continent that in certain years when the climate has been unusually erratic, the flight-year has been suddenly either a year late or a year early; it then returns to its usual cycle. Further, as an example of this irregularity in this country, the flight-year for *Melolontha* in the Forest of Dean was due according to the ordinary cycle to occur in 1935, but an immense flight took place in 1934, a year preceded by a very dry and warm summer and winter.

Another point which may help to explain the absence of large and regular flight-years of *Melolontha* in the British Isles is the very marked difference in the weather of the spring on the Continent and in this country. Thus over large areas of the Continent the weather changes very rapidly from winter to spring, the temperature rising suddenly in a few days, conditions which no doubt tend to bring out all the *Melolontha* at about the same time and thus form a very marked flight. In England, on the other hand, the change is much more gradual.

The flight-years of *M. melolontha* in southern England seem to recur roughly every 3 or 4 years, while those of *Amphimallus*, which are considerably less well marked, take place every 2 or 3 years. With *Serica* there is little indication of a flight-year, as is to be anticipated, since development occupies only 2 years.

Summary

1. Adult chafer beetles do not usually occur in very extensive swarms in the British Isles, and their general importance lies in the characters of the larvae which are notable for their voracity and large size. Another factor of importance is the tendency towards concentration, large numbers of beetles often ovipositing in one field while neighbouring crops remain free.

2. There are few crops which the larvae do not attack, but the damage done by the adult beetles in this country is not important.

3. The distribution, which is local, is shown to be controlled by the sparseness of suitable habitats and by the prevailing conditions of temperature and humidity.

4. The factors which control the varying lengths of the life cycle are also considered to be mainly temperature and humidity. Thus, although *Serica* always takes 2 years to develop, *Amphimallus solstitialis* may take either 2 or 3 and *M. melolontha* 3 or 4 years, according to the conditions controlled by these two factors.

5. Regular flight-years are usual on the Continent, but are much less definite in the British Isles, and the numbers of beetles appearing are relatively small. It is suggested that the reason for this is the differences in climatic conditions, particularly in spring.

The writer wishes to express his thanks to Mr W. E. H. Hodson, the Advisory Entomologist at Reading University, under whose kind supervision the work was carried out, and also to Mr J. M. B. Brown of the Forestry Commission, for generously supplying numerous specimens of *Serica* larvae and much information.

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A SENSITIVE AND EASILY MADE WATER-COOLED DEW-POINT INDICATOR

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(With 3 Text-figures)

Originally designed for use in a constant humidity chamber, the apparatus to be described has proved so satisfactory that it is hoped that the following description of it may be found useful.

Unless specially adapted, the instrument is not sufficiently portable for field work, but for general laboratory or biological work in breeding cages it possesses several advantages over the more conventional ether-cooled type.

Not the least of these in biological work is the freedom from possible leakage of ether vapour, unsatisfactory sealing of the metal thimble to the glass tube of the ordinary bubbling type of instrument being a common source of trouble. Moreover, with the latter the temperatures registered by the thermometer at the appearance and disappearance of the dew film are quite widely divergent, the mean value being taken for an approximation to the true dew-point.

With the water-cooled instrument to be described, it is possible to hold the temperature steady when the dew-point has been reached, and by slow adjustment of the cooling stream to get the temperatures of the appearance and disappearance of dew film very much closer together. The appearance of dew film may be observed visually, or by any of the more elaborate optical or photo-electric methods.

The instrument itself consists of a small metal box, just large enough to accommodate the bulb of a good mercury thermometer, and the ends of narrow inlet and outlet pipes. One face of the box is replaced by a thin copper diaphragm, on the brightly plated outer surface of which the formation of dew is observed. The correct design of the inlet pipe is important.

The cooling operation consists of drawing a slow stream of cold water through the instrument continuously by means of a small aspirator. Temperature is adjusted by mixing a variable proportion of water at room temperature with the cold stream. Freezing mixture may be used if very low humidities are to be measured, but a mixture of ice and water suffices at normal room temperatures for humidities down to 35 per cent. (*vide* dew-point tables).

In practice the rate of flow of the cold water is fixed by an initial adjustment of the "cold" valve, which may consist of a screw pinch-cock on thick-walled rubber tubing. The addition of water at room temperature must be adjusted by means of a good valve of some sort—either a pin valve or a tap with a tapered groove round its inner cone. If a good valve is used, the regulation of temperature will be found to be surprisingly easy and critical. The author uses a tapered tap which was originally intended for an oxygen flow regulator, and finds no difficulty in holding the temperature of the dew-point box steady to 1/10° C.

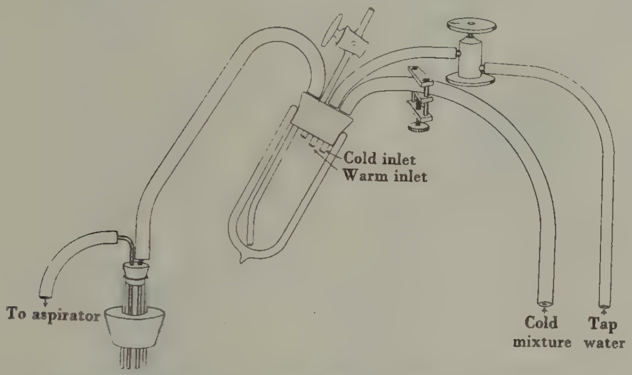

Fig. 1. The cooling system. The Dewar flask mixer is drawn in section to show the arrangement of inlet and outlet pipes.

To facilitate the adjustment of the rate of flow of both the cold and warm water, and to ensure proper mixing, a small unsilvered Dewar flask is included in the circuit. Into this is fitted a soft rubber bung through which pass four glass tubes. One projects to the bottom of the flask to suck the mixed water to the dew-point box. Two form the inlet of the cold and warm streams. They project about half an inch below the bung, and from them the two streams drip into the flask which is tilted slightly so that both streams fall on the side and run down mixed as a single stream. The fourth is a tap communicating with the air, the purpose of which is to enable any water which has collected at the bottom of the flask by back pressure when the aspirator is turned off, to be drained out on starting up. It is desirable to have as small a reservoir at the bottom of the flask as possible, otherwise an adjustment of the flow tap will take some time to alter the temperature in the dew-point box.

The ice and water mixture may conveniently be sucked from a

Thermos bottle through thick-walled rubber tubing. The tube connecting the Dewar flask with the inlet pipe of the dew-point box must also be thick-walled in order as far as possible to prevent gain in heat by conduction from the air.

The aspirator may take the form of a separating funnel, as when once filled it is replenished by the outlet from the dew-point box, incidentally providing a further indication of the total rate of flow.

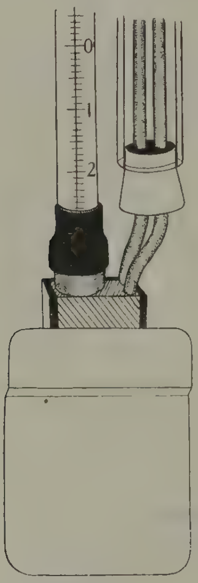

Fig. 2.

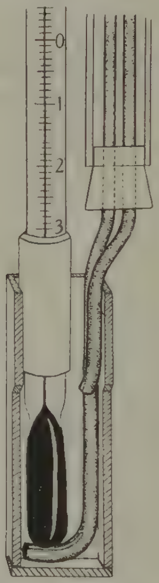

Fig. 3.

Fig. 2. Dew-point box completed.

Fig. 3. Dew-point box before fitting diaphragm. Thermometer in position. Note the slit along the foot of the inlet pipe.

The manufacture of the dew-point box presents no difficulties, provided the ordinary workshop facilities are available, with the exception of the chromium plating of the diaphragm face. This plating costs about eighteen pence.

A good thermometer graduated in tenths of a degree is advisable, and for the highest degree of accuracy should have been calibrated for a depth of immersion of $1\frac{1}{2}$ in.

The other materials required are as follows.

The thermometer bush. A 1 in. length of fairly thin-walled copper or brass pipe, through which the bulb and part of the stem of the thermometer fit reasonably well.

Inlet and outlet pipes. Two lengths of narrow copper tubing of the type used in model engineering. An inside diameter of a little over a sixteenth of an inch is suitable. In the writer's case it was found convenient to instal the instrument with the whole thermometer within a standard humidity chamber, and accordingly the inlet and outlet pipes were first cut about 6 in. longer than the overall length of the thermometer. If it is desired to instal the instrument with the stem of the thermometer projecting above the mounting bung, the pipes can be correspondingly shorter. In the latter case it must be borne in mind that it will be necessary to have a thermometer so calibrated that the zero mark will project well above the mounting bung.

The diaphragm. A piece of thin copper sheet 2 in. square, as thin as consistent with rigidity when soldered on. About the thickness of good quality notepaper will be found satisfactory.

The box. A piece of channel section brass of an internal width sufficient to accommodate the thermometer bush and the inlet and outlet pipes. Half an inch will probably be found sufficient.

A $2\frac{1}{4}$-in. length of this has the sides cut down so that the bush fits flush with the sides. The sides are now further cut or filed down, starting $\frac{1}{2}$ in. from one end, until the depth of the channel equals the diameter of the bush less the thickness of its wall. When the thermometer is pushed through the bush and the latter held in position in the channel brass to the extent of $\frac{1}{2}$ in., the thermometer bulb should lie flush with the recessed part of the channel brass. If the diaphragm is placed on the channel brass, it will just touch the thermometer bulb.

One of the thin copper pipes is bent sharply at one end to fit in the manner shown in the diagram. The end is closed by tapping over with a hammer or by solder, and a fine slit is filed along the side of the bent portion. The slit should be $\frac{1}{2}$ in. long, and its area approximate to the bore of the pipe. It is an important refinement and materially assists the uniform distribution of temperature.

The sides of the channel brass are cut away to an extent sufficient to enable the projecting bit of the back to be bent upwards and soldered to form the end of the box.

The pipes are now fitted and bent in the manner shown, the whole being filled in with solder to the level of the end of the bush and outlet pipe. During this operation it is advisable to plug the end of the pipes to prevent solder from getting into them. After removing the plugs the diaphragm may be soldered on, first tinning it on the inner surface where the edges of the box meet it, but not elsewhere.

After trimming the diaphragm to project ½ in. on each side of the box and fitting a suitable length of wide-bore glass tubing to insulate as much as possible of the pipes, the instrument is ready for chromium plating. After this the thermometer can be fitted and held in place by a piece of rubber tube over the neck of the bush.

The insulating glass tube can now be passed through the mounting bung, and after connecting the cooling apparatus the apparatus is ready for use.

The operation is as follows. The thick-walled tube leading to the screw pinch-cock and cold inlet to the Dewar flask dips into a Thermos bottle containing the cold mixture. The tube leading to the fine adjustment valve dips into a vessel containing tap water. The outlet from the dew-point box is connected to the aspirator, which is filled and the tap turned on. The cold pinch-cock is screwed off, and the warm fine adjustment valve opened until the tap water is dripping into the Dewar flask at a speed of about six drops a second. When the apparatus is full of water and bubbles cease to pass into the aspirator, the cold valve is slowly opened until both streams drip into the Dewar flask at the speed of about four drops a second. The dew-point temperature is now controlled by the fine adjustment valve only.

If the total rate of flow is kept as slow as possible consistent with the attainment of the dew-point temperature, it will be found that dew forms evenly all over the face of the diaphragm. Moreover, the cooling mixture lasts longer. If the cooling is too rapid, dew first forms in a patch opposite the slit in the inlet pipe, at the bottom of the box. With very little practice it is possible to attain and hold the dew-point temperature quickly and easily, the temperatures at which dew forms and disappears differing by not more than 0·5° C.

As there is a continuous flow through the box, a thin diaphragm of high thermal conductivity, and the thermometer bulb in close approximation to the diaphragm, the results should compare favourably with those given by accurate thermocouple measurement of the temperature of the diaphragm.

In practice the instrument gives readings which are of more than adequate accuracy for most purposes, enabling the relative humidity to be read from the dew-point tables plotted to large scale, to well within 1 per cent.

(*Received October* 10, 1935)

PROCEEDINGS OF THE ASSOCIATION OF APPLIED BIOLOGISTS

General Meeting of the Association of Applied Biologists held in the Botany Lecture Theatre of the Imperial College of Science and Technology, South Kensington, London, on Friday, November 15, at 2.30 p.m. The President, Dr T. Goodey, in the Chair.

The following papers were read on "Insect Population Studies".

I. Insect Fluctuations: Population Studies in the Gall Midges (Cecidomyidae). By H. F. Barnes, M.A., Ph.D.

II. Fluctuations of Insect Populations: Field Observations. By A. Roebuck, N.D.A.

I. INSECT FLUCTUATIONS: POPULATION STUDIES IN THE GALL MIDGES (CECIDOMYIDAE)

By H. F. Barnes, M.A., Ph.D.

(*Entomology Department, Rothamsted Experimental Station, Harpenden, Herts.*

Introductory and methods

These studies were designed in the first place to obtain quantitative data regarding the fluctuations in numbers of insect pests in the field. This of necessity included the study of the variation in numbers of the pests' parasites as well as of the fluctuations in the numbers of the pests themselves. In addition the varying extent of damage or infestation of the crop was taken into account. Secondly, these studies were designed to indicate possible factors involved in these fluctuations. Broadly speaking, the main factors concerning which information was required were (*a*) biological, such as the role played by parasites, and (*b*) physical, such as the effect of weather conditions.

The need for such a series of studies was emphasised by the tendency to draw a rather abrupt line of distinction between the phenological method of approach to the study of populations and the more academic study of populations under controlled conditions. What were and still are really needed are sets of data concerning the fluctuations in numbers of the more important pests extending over a long period of time, comparable with the data one can obtain regarding the numbers of certain fur-bearing animals and certain game birds. These data also should be amenable to statistical treatment. In addition, the indication of possible factors controlling the fluctuations would be of great importance and help to laboratory workers.

Six species of gall midges were chosen in 1927 for the initial studies. This choice was chiefly a matter of convenience. It was decided to study each insect for a period of about 5 years and then to continue studying only those that were giving the best results. The gaps thus made were to be filled by substituting other well-known insect pests belonging to other orders. This stage in the work was reached in the spring of 1934, and since that time other insects have been considered with a view to incorporating them into the study. Difficulty, however, has been experienced in selecting other insect pests, because, strange as it may appear to some, there are so many obvious discrepancies in our knowledge concerning the exact biology of many of our common insect pests. It will be clear later how important a detailed knowledge of the biology is in such population studies, especially in deciding the best time to sample and the kind of sample to take. In fact the methods to be adopted have their foundation on the biology of the insect in question.

Parallel studies pursuing further the hints of possible factors involved in the fluctuations were intended to accompany and to supplement the main investigation.

The gall midges chosen were as follows: *Contarinia tritici* Kirby and *Sitodiplosis mosellana* Géhin, the two wheat-blossom midges; *Rhabdophaga heterobia* H.Lw., the button-top midge of basket willows; *Dasyneura alopecuri* Reuter, one of the three midges known to prevent seed formation in meadow foxtail grass; *Dasyneura arabis* Barnes, the Arabis midge; and *Dasyneura pyri* Bouché, the leaf-curling pear midge.

The methods adopted were briefly the collection and counting of the full-grown larvae from crop samples of known size. This provided figures for the infestation of the crop. Then the larvae were reared under as nearly natural conditions as possible. Subsequently the adult midges and their parasites emerged, thus giving the date of emergence and figures from which the degree of relative parasitism could be calculated. The relative parasitism is a term used to denote the proportion of parasites emerging compared with the corresponding number of host midges. Such figures give some idea of the potential insect attack after the turmoils and troubles of the periods of development, especially the winter, have been passed. An obvious elaboration of the investigation is the comparison of the parasitism at different stages in the development of the pest and the differential effect of conditions on the host and parasite. This is the type of parallel or subsidiary study one has attempted to carry on.

The particular species of midges selected were of diverse nature as regards number of generations, position on the plant they attacked, whereabouts of the over-wintering larvae, and so on. In addition the samples of each midge were gathered from different parts of the country. The wheat midges were collected on Broadbalk field at Harpenden, the button-top midge from a commercial willow bed at Syston, near Leicester, the meadow foxtail midge from near Aberdeen and the leaf-curling pear midge from a garden near Barnstaple in Devon. They were all reared at Harpenden. It would have been better to collect and breed samples of each midge in several localities, but this was impracticable. Such a study as this should be carried out by a team of workers, but much of the labour entailed in collecting data is purely routine and consequently unattractive. Furthermore, few workers have sufficient leisure to do such long period studies.

The data

The results so far obtained are promising. The numbers of larvae of the two wheat-blossom midges present in 500 ears on Broadbalk since 1927 are shown in Table I.

It is obvious that there have been large fluctuations in the numbers of larvae present. The extent of relative parasitism also has varied considerably. In the case

Table I

Numbers of larvae of Contarinia tritici (*A*) *and* Sitodiplosis mosellana (*B*) *present in* 500 *ears of wheat on Broadbalk*, 1927–35

	1927	1928	1929	1930	1931	1932	1933	1934	1935
A	1,780	2,195	19,265	18,595	19,273	7,356	1,511	3,381	4,289
B	715	2,043	587	3,748	6,027	3,114	319	572	4,221

of *Contarinia tritici* the lowest figure has been about 10 per cent. and the highest between 70 and 80 per cent. It has been suggested that more than 50 per cent. relative parasitism may be effective in reducing the midge population. The other wheat-blossom midge, *Sitodiplosis mosellana*, has also varied both in numbers and in relative parasitism. The latter in this case has varied from 30 to 85 per cent. It has been suggested that more than 80 per cent. relative parasitism may be effective. The numbers of grains attacked shows similar fluctuations. Table II shows the percentage numbers of grains attacked.

Table II

Percentage of grain attacked by the larvae of Contarinia tritici (*A*) *and* Sitodiplosis mosellana (*B*) *on Broadbalk*, 1927–35

	1927	1928	1929	1930	1931	1932	1933	1934	1935
A. Percentage lost grain, *C. tritici*	0·95	0·79	5·9	5·9	6·4	4·9	0·7	1·5	2·1
B. Percentage shrunken grain, *S. mosellana*	2·2	5·7	1·8	11·7	15·0	10·5	1·4	2·5	18·0

The larvae of *Contarinia tritici* prevent the formation of a grain and live on an average about 9, 10 or 11 larvae per floret, the maximum number found being as many as 91. On the other hand, the larvae of *Sitodiplosis mosellana* cause shrunken grain and live on the average about 1 larvae per grain, the maximum number being 12. Whether the figures of grain attacked bear any relationship to the extent of damage is a debatable point, since there may be a compensation before harvest or even a general loss of weight in grain throughout an attacked ear.

If both species are considered together, one sees that the year 1927 was notable because of the low numbers of larvae as was also the year 1933. Further, a definite peak in population was reached in 1931 when there were over 25,000 larvae present in the 500 ears of wheat. Taking advantage of the Ministry of Agriculture's monthly *Reports* as to the prevalence of insect pests, which are based on eye observations by experienced entomologists, one was able to see three previous years when the wheat midges were very abundant. These years were 1916, 1920 and 1926. Similarly there were other years when the midges were more scarce than usual. With this information a hypothetical curve of abundance was drawn covering the years 1915–26, and to this was added the curve obtained for the years 1927–32 inclusive by the direct counting method. One was then tempted to find a rhythmic fluctuation in numbers and predict in the autumn of 1932 that there would be another peak in numbers about the year 1937. Fig. 1 shows the numbers of wheat-blossom midge larvae separately and collectively. In addition the relative parasitism of the two species is shown. It can be seen that the peak of parasitism lagged behind that of the numbers of midges, and that now the numbers of both species are increasing while the parasitism is reaching a low level.

The numbers of button-top midges (*Rhabdophaga heterobia*) and its parasites reared from 500 galls of the over-wintering generation show interesting fluctuations.

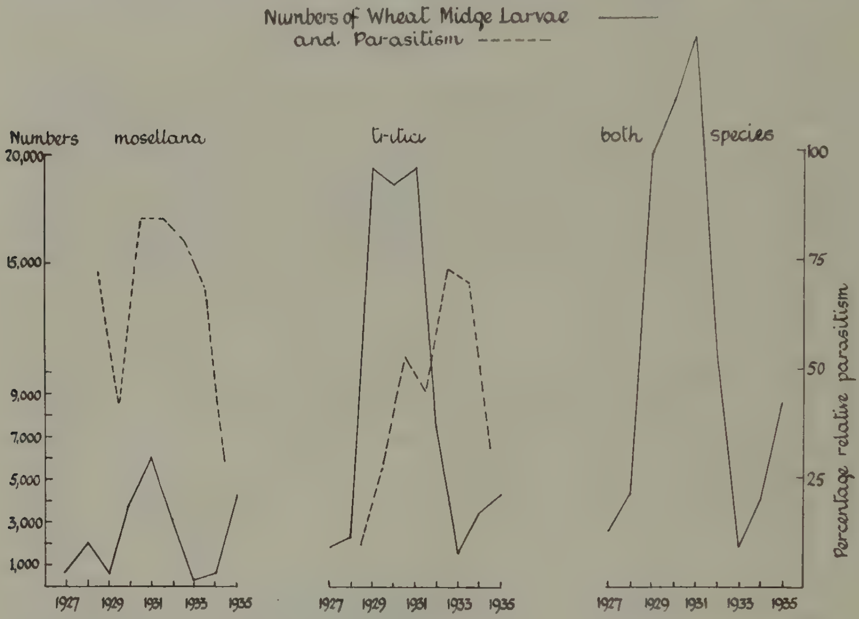

Fig. 1.

Table III shows the average population of 500 galls in the years 1928–35. It is seen that in 1928, 1929, 1932 and 1933 the insect population was about 3000. This is taken to be the normal population that emerges in the spring from 500 galls. In contrast to this in 1930 only about 1000 insects emerged and in 1931 only about 2000. It has already been claimed that this sudden drop in population was due to a drought in the summer of 1929 which resulted in a serious stunted growth of the willows the next

Table III

Average population of 500 *galls of* Rhabdophaga heterobia, 1928–35

Year	Midges	Parasites	Total insects	Percentage relative parasitism
1928	1573	1607	3180	51
1929	1235	2204	3439	64
1930	341	556	897	62
1931	840	1323	2163	61
1932	1480	1662	3142	52
1933	2810	428	3238	13
1934	796	633	1429	44
1935	234	148	382	38

year. It was not till 1932 that the willows grew to their normal height, and similarly it required 2 years or about six generations for the insect population to get back to the normal 3000. Again, the summer of 1933 was exceptionally dry, as was also that of 1934, the reduction of the insect population in 1934 and again in 1935 may be the reflection of these dry summers. The sudden drop in relative parasitism in 1933 and corresponding rise in numbers of midges may be due to a weather factor acting differentially on the host and parasite. A similar sudden increase in midges accompanied by a fall in number of parasites was recorded in the study on *Dasyneura alopecuri*. In this case every generation of the insect was under investigation. The parasite, in the brood previous to this change in relative parasitism, had emerged slightly in advance of the host midge and so presumably were unable to pursue their normal attack on the midges' larvae.

The studies on the foxtail, arabis and leaf-curling pear midges were allowed to lapse after the initial study, but they each gave interesting results as can be gathered from the discussion.

Discussion

In discussing results such as these it is of importance to examine what periods during the life of the insects in question are especially dangerous to the multiplication of the race. In other words, what are the odds on the insects reaching maturity when once they have reached a definite point in their life cycle?

For the sake of following this line of thought it is convenient to divide the life of the insect into four main periods, namely: that of emergence, that of egg laying, that of growth and lastly that of development.

Period of emergence.

Dealing with emergence, these studies have brought out several points. In the first instance, *the time of emergence of the midges compared with that when the plant to be attacked reaches the stage of growth necessary for optimum oviposition* is important. In other words, the adjustment between plant and insect. For example, the wheat-blossom midges oviposit in the ears of the wheat as soon as the latter burst from their enveloping sheaths. The midges cannot lay their eggs before this takes place, and, in addition, they live only about 24–48 hours. In 1933 an abnormally warm spring followed a warm winter. The wheat ears burst from their sheaths 2 weeks earlier than normal, but the wheat midges emerged 3 weeks earlier than is usually the case. The result of this lack of exact adjustment of plant and insect was that probably the midges oviposited largely on their alternate host plants. But in any case the numbers of larvae subsequently found in the wheat ears showed a great drop when compared with those of the previous year. In 1932 there were 7356 larvae of *Contarinia tritici* compared with only 1511 in 1933; similarly in the case of *Sitodiplosis mosellana* the corresponding figures were 3114 and 319.

Secondly, *the time of emergence of the midge compared with that when the parasites emerge* may cause a definite change in population. Normally the host midge will emerge before its parasites. But when studying *Dasyneura alopecuri* it was noticed that in 1928 the parasites emerged earlier than usual in relation to when the midges emerged. In fact the crest of emergence of parasites was about 1 week in advance of that of the midges instead of about 3 weeks later as is normal. In this year 1588 midges and 979 parasites emerged from 100 heads of grass giving the relative parasitism of 38 per cent. The following year, 1929, 4748 midges and only 114 parasites emerged from the same sized sample. This gave the relative parasitism as 2·3 per cent. The great reduction in number of parasites and corresponding increase in midges were probably due to this change over in relative times of emergence of host insect and parasites.

Thirdly, *the sex ratio at the time of emergence* must be considered. These studies have shown that there are definite sex ratios at emergence for different species of gall midges. For example, when large numbers are considered, *Contarinia merceri* gives 23 : 77, *Dasyneura arabis* 23 : 73, *D. pyri* 39 : 61 and *D. alopecuri*, *Contarinia tritici* and *Rhabdophaga heterobia* 50 : 50. On the other hand, when isolated females of *R. heterobia* were allowed to oviposit and their progeny reared, it was discovered that they breed by means of unisexual families. This means that the progeny of one female are either all male, all female, mostly male with an occasional female, or mostly female with an occasional male. Further, it has been shown that the sex ratios of the different generations of *Dasyneura arabis* and *D. pyri* are different (Table IV). In *D. arabis* as the season advances so the percentage of males decreases, *i.e.* the first generation of the year has the highest percentage of males, the second generation less, the third generation less still and the over-wintering generations the least. In *D. pyri* the reverse is the case, as the season advances so the percentage of males increases. In this connection it is interesting to note that experiments have been carried out which prove that delayed mating in *D. arabis* tends to result in the production of more males than is usually the case.

Table IV

Sex ratios of different generations of Dasyneura arabis *and D. pyri*

	1st generation	2nd generation	3rd generation	Over-wintering generations
D. arabis	43 : 57	26 : 74	21 : 79	16 : 84
D. pyri	32 : 68	36 : 64	38 : 62	47 : 53

Besides these differences in sex ratios which are claimed to be the normal ones, examination of the data reveals that there have been two types of abnormal sex ratios. For instance, there has been an occasion when the sex ratio of *D. alopecuri* was 25 : 75 instead of the normal 50 : 50, and on another occasion the sex ratio of the over-wintering generations of *D. arabis* was 3 : 97 instead of the average 16 : 84. This can be considered as an abnormality in the total or final sex ratio of the generation. The other type of abnormality has been in the proportion of the sexes emerging on individual days. In this case the alteration in sex ratio may be temporary only. Some evidence exists to show that in *Rhabdophaga heterobia* and *Dasyneura alopecuri* the females are more subject to non-lethal factors than the males as regards emergence but that the males are more sensitive to lethal ones. Heat and cold appear to act differentially on the sexes.

Fourthly, *the dates of emergence and number of generations* are noteworthy. The constancy of the emergence periods of the over-wintering generations is remarkable. But in an early year more midges start to emerge earlier. These early emergences are not so regular in approaching the peak as are the later ones and are liable to be influenced more by day to day changes in the factors effecting emergence. The view is held that the larvae require a certain more or less fixed amount of temperature to develop from fully fed larvae into adults. When such amount of temperature has been made available, then under given favourable conditions from day to day emergences will take place. On the other hand, although favourable day to day conditions may occur before such amount of requisite temperature has been received, emergences will not occur.

The number of generations also is remarkably constant. The chief effect of a warm season is to advance the emergence dates of the generations but not to increase their number. A further effect is an alteration in the proportion of the later generations which carry over to the next season.

The egg-laying period.

The next possible danger period is that of oviposition. The weather conditions prevailing immediately after the midges have emerged are important in this connection. Windy weather, for instance, can be very deleterious to successful oviposition in the case of the wheat-blossom midges. As the midges are so short lived two unfavourable days at the peak of the emergence period would cause a serious diminution in numbers of eggs laid.

A second way in which the weather can effect the population at this stage is the effect it has on the relative speeds of plant growth and the insect egg development. An example of this is as follows. The midge *Macrolabis corrugans* F.Lw. very commonly attacks *Heracleum Sphondylium*, the common weed, cow-parsnip or hog weed. The white larvae are to be found in the galled leaves which remain crinkled and finally blacken. This same midge has been known to attack the cultivated parsnip in Kent in 1918, in Yorkshire in 1927 and near Edinburgh in 1932. It seemed curious that so common an insect should attack the cultivated parsnip so rarely if indeed it were the same species. Experiments proved that there was only one species of midge involved. Further, they demonstrated the fact that the leaves of cow-parsnip unfurled much slower than those of parsnip. The result was that, although the midges laid their eggs just as readily on both plants, the eggs laid on parsnip were very soon exposed on the opened leaf where both they and the very young larvae perished. On the other hand, those on cow-parsnip remained hidden in the crevices of the unopened leaves and so could develop. In cases where the conditions for plant growth of the parsnip were unfavourable, the speed at which the leaves uncurled was lowered and then there was a chance for the larvae of the midge to establish themselves on the parsnip leaves, unless the conditions which were disadvantageous to the plant were also deleterious to the development of the eggs.

The growth period.

When once the larvae of gall midges have safely reached the stage when they are within their galls they are to some extent free from many dangers. However, they are dependent upon the plant for nourishment. If anything seriously affects their host plant, the midges are liable to suffer. Drought, for example, apparently has seriously affected the numbers of the button-top midge (*Rhabdophaga heterobia*) owing to there being a stoppage of growth and consequently a shortage of movement of sap within the plant. Such a calamity, however, affects the midges' parasites as well as the midges themselves.

There is another less obvious danger to the insect population. When the conditions for insect growth are really favourable late in the season a larger proportion than normal of the last brood of the midges emerges the same year. Unless the period of plant growth is extended, which is frequently not the case in a hot year, the midges emerging so late in the year cannot continue their race. If indeed they can oviposit safely the chances are that the larvae cannot complete the growth period either before the plant ceases to supply their needs or the weather precludes further development. In such cases it may be claimed that a warm and late summer can be of serious disadvantage to a midge population, as the late emergences would be biological waste. One species in particular seems to suffer in this way, *i.e.* the leaf-curling pear midge.

The period of development.

After the larvae have become fully grown there is either the pupal stage or the winter to pass as fully grown but undeveloped larvae. Often this period is spent in the soil. In attempting to rear such larvae one usually has been successful in breeding to the adult stage rather less than 50 per cent. of the larvae. On the other hand, one breeds well over 90 per cent. of the larvae which pass the winter in the plant galls. Whether this shows a lack in ability to rear the insects or that the soil is a dangerous medium for the larvae is open to question. But it seems likely that a certain number of the larvae in the soil would normally perish during the winter and also just at the emergence period of the adults in the spring.

Finally, throughout these periods of emergence, oviposition, growth and development there is the danger from parasites and predators. One exceptionally interesting fact regarding parasitism of the leaf-curling pear midge has arisen, namely, the pear midge develops at a quicker rate than the parasite *Misocyclops marchali* Kieffer. The result is that the first brood of the year escapes attack. In each of 3 years this brood was free from parasitism, and during this period each of the other broods was parasitised. Dumbleton working on the same midge and parasite in New Zealand had similar results.

Summing up, it is obvious that, broadly speaking, weather is the underlying set of factors controlling increases or decreases in gall-midge populations. The weather acts in three ways. Firstly, on the insects themselves, thus influencing the sex ratio at emergence, the dates of emergence, number of broods and the activity of the adults during the ovipositional period. Secondly, it acts on the insects through the plants. Examples of this are effect of drought periods, alterations in length of life cycle and the effect of lack of close adjustment between plant growth and insect development. Thirdly, the weather acts on the insects through their parasites. In this division one has given the example of the differential effect of weather on the length of life cycle of host and parasite. In addition there is some evidence to show that coldness and warmth acts differentially as regards its lethal qualities on host midge and parasite, as well as on the two sexes of the midges.

In conclusion, accounts of this investigation on populations of gall midges have been published from time to time since 1932 in the *Journal of Animal Ecology*. Full references can be found there. The last of the five studies, as well as a general discussion of the whole investigation, has been published in that journal this month (November 1935).

II. FLUCTUATIONS OF INSECT POPULATIONS: FIELD OBSERVATIONS

By A. ROEBUCK, N.D.A.

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The possibility of great changes in numbers in various species of insects from year to year is well known to field entomologists. The difficulty is the measurement of populations.

There is no standard *method of sampling*. It is almost necessary to work out a method for each species studied. In some cases egg counts are possible—this is the ideal method. In others larvae, pupae or adults have to be counted. In many cases a colony or nest is the best unit for measurement. The numbers of some species are best estimated by their effects on crops, *e.g.* destroyed tillers, damaged seeds, etc. Where there is more than one generation a year it is often impossible, owing to overlapping, to count each generation. When the life cycle extends over several years, it is often desirable to separate the successive generations.

There is always the possibility of complication through the question of host plants. A species with a single host plant is the most easily studied. Where there are several host plants, for various reasons, one or other may be preferred in any one season. When wild plants are alternative hosts the difficulties are further increased.

When a method of sampling has been adopted there arises the greater problem of *the area to be sampled*. One would like to know the populations over the whole country, but being restricted to a province, in this case of five midland counties, this becomes the maximum area for study. The local distribution of many species often further limits the area to be studied. The easiest to study are those species whose host plants are in restricted areas and are long lived plants, *e.g.* trees.

A great difficulty with farm pests is that the crops change position yearly and the insects have, of necessity, to move also. Local migrations and large scale migrations may disturb results. There may be also special local concentrations of particular species.

The ultimate aim is to estimate the summation of the various factors affecting the numbers—the various climate factors, parasites, predators, diseases, etc.

In studying populations one finds two series of factors involved. Certain insects are scarce for years and then suddenly increase in numbers. What are the factors which produce these sudden irruptions? On the other hand many species are common and abundant for many seasons and suddenly become scarce. What are the factors which cause these rapid diminutions?

The following species are given to represent both these types.

Examples of sudden irruptions

(1) *Andrena fulva* (lawn bee).

In 1925 there were 153 nests on the lawns in our garden in Leicestershire. In 1923, 1924, 1926 and all subsequent years to 1935 the number of nests a year have been 0 to 6. This is possibly not important. It may be a habit of this species to concentrate on a piece of ground in any one year and avoid that ground for many subsequent years.

(2) *Blennocampa pusilla* (leaf curling sawfly of the rose).

In 1931 this species was abundant everywhere throughout the Midland Province. The number of individuals was enormous. In the years 1922–30 and 1932–5 it was rare. Many species of sawflies have this habit of periods of rarity suddenly broken by a period of extraordinary abundance.

(3) *Trochilium apiforme* (hornet clearwing of the poplar).

This is normally a rare species. In a large garden surrounded by poplars in Nottinghamshire there appeared large numbers in 1932. These were from eggs laid in 1930. They were not noticed previously. By counting the emergence holes the population was (in round numbers):

1932	500
1933	1000
1934	750
1935	200

A complication has arisen which will affect the insect. A number of the trees have already been cut down because of silver leaf attacks and quite a number of others are infected.

The garden is quite isolated by built up areas and there is every reason to believe that the colony of insects is unaffected by outside influences.

(4) *Hylemyia brunnescens* (carnation fly).

This normally attacks carnations. The larvae are stem borers. An outbreak occurred near Leicester in the autumn of 1934 where the larvae were leaf miners in Sweet Williams (*Dianthus barbatus*). A number of adults were reared in the laboratory and puparia were collected in the garden, and from these more adults were reared. Over 100 adults of both sexes were supplied with more Sweet William plants but no eggs were laid. They have also disappeared from the garden.

(5) *Psylliodes chrysocephala* (cabbage stem flea beetle).

This insect appeared in immense numbers in the cabbage plant growing district of the Charnwood Forest area of Leicestershire during the winter of 1934–5. Previously collectors have obtained specimens of the beetle in Leicestershire but it has not been a pest (since 1922). A field which should have cropped 3,000,000 plants had the crop completely ruined by the larvae. From counts made in April 1935 it was estimated that there could not have been less than 3,000,000 larvae in the field. The total population on three other fields was estimated to be not less than 5,000,000. The adult beetles emerged in June. The whole attack, so far, is in a well defined area and it is hoped to study these insects further.

Examples of more stable species

(6) *Phyllodecta vulgatissina* (blue willow beetle).

This species is restricted to the *Salix viminalis* varieties. It was studied on an isolated block of willows about 4 miles north of Leicester on the banks of the River Soar, with check areas north and south of this block.

The number of beetles increased in 1919 and 1920 so that it became a serious pest and skeletonised the crop in 1921 and subsequent years. The adults can be counted on the young shoots during the first week of June and the larvae about the middle of July. The graph shown was based on counts of adults, as the larval counts are not complete for the series of years. Larval counts are roughly five times as large as the adult ones. The adults numbered:

In 1922, 13 per rod or 1,600,000 per acre.
In 1923, 12 per rod or 1,500,000 per acre.
In 1924–9, 8–12 per rod.
In 1930, 7 per rod or 875,000 per acre.

In 1931 and subsequent years it has been difficult to find individual specimens in the area. The larvae appear to have all perished during the wet weather in July 1930.

(7) *Psila rosae* (carrot fly).

The results given apply to field crops on the east side of the valley of the River Trent, partly in Lindsey and partly in Notts. Of about 2500 acres grown over 1000 acres are examined. This species has two generations a year. Larvae of the first generation are sampled at the beginning of July. Larvae of the second generation can sometimes be counted at the end of October but check counts should be made later, say January and March.

The following are the larval counts:

In 1925 (1st gen.) 10 per sq. yard or 50,000 per acre.
1926 (1st ,,) 8 ,, 40,000 ,,
1927 (1st ,,) 20 ,, 100,000 ,,
,, (2nd ,,) 30 ,, 150,000 ,,
1928 (1st ,,) 16 ,, 80,000 ,,
,, (2nd ,,) 16 ,, 80,000 ,,
1929 (1st ,,) 12 ,, 60,000 ,,
,, (2nd ,,) 30 ,, 150,000 ,,
1930 (1st ,,) 12 ,, 60,000 ,,
1931 (1st ,,) 60 ,, 300,000 ,,
,, (2nd ,,) 40 ,, 200,000 ,,
1932 (1st ,,) 24 ,, 120,000 ,,
,, (2nd ,,) 12 ,, 60,000 ,,
1933 (2nd ,,) 8 ,, 40,000 ,,
1934 (1st ,,) 4 ,, 20,000 ,,
,, (2nd ,,) 12 ,, 60,000 ,,
1935 (1st ,,) 2 ,, 10,000 ,,
,, (2nd ,,) 1 ,, 5,000 ,, (provisional).

(8) *The chafer beetles.*

Four species have been under observation in Lincolnshire and Nottinghamshire. They are very striking during "flight" periods.

(*a*) *Rhizotrogus solstitialis* (summer chafer). The last recorded swarming or flight period was 1901 in Lincolnshire. It is on the whole a scarce species and has certainly been so since 1922.

(*b*) *Serica brunnea* (brown chafer). This species swarmed in 1910 in North Lincolnshire. Since then there have been few about.

(*c*) *Phyllopertha horticola* (garden chafer). This species has been studied in central Nottinghamshire, in the Sherwood Forest district where, at times, it is a serious pest to the grassland. The area of land specially kept under observation is approximately 40 sq. miles. The normal population of larvae since 1928 has been 1 per sq. yard or 5000 per acre (3 millions per sq. mile). In 1928 numbers greatly increased. The population in the bad portions average 9 per sq. ft. or 81 per sq. yard or 392,000 per acre or 250 millions per sq. mile. By 1931 the numbers had increased to 12 per sq. ft. or 108 per sq. yard or 520,000 per acre or 320 millions per sq. mile. In places the numbers were 24 per sq. ft. or over 1 million per acre. The area occupied by the very bad attacks was approximately 1/40 of the area studied. The graph shown was prepared by superimposing the dense population on the normal and averaging this for the whole area. It will be noticed that the severe outbreaks lasted from 1928 to 1931. It is interesting also to note that the records of a farmer in the middle of the area showed that the same fields were attacked in 1898 and following years, that is 30 years previously.

(*d*) *Melolontha vulgaris* (cockchafer). Whereas the life cycle of *Phyllopertha* is completed in 1 year that of *Melolontha* appears to take 4 years in Nottinghamshire. The problem of abundance or scarcity is complicated in this species by the fact that the larvae are practically all of the same age in any one year. This results in the adults appearing in numbers one year and absent for the next 3 years. The population study, therefore, since 1928 has virtually resolved itself into a life-cycle study. The area studied was a little north of the *Phyllopertha* district. Arable crops are commonly attacked. 1928 and 1932 were pest years, when the larvae were destructive (2–5 per sq. yard or 10,000 to 25,000 per acre). Smaller larvae were abundant in 1931 and 1935 (4–8 per sq. yard = 20,000–40,000 per acre). 1930 and 1934 were flight years for the adults when eggs were laid. 1929 and 1933 were resting years. There is a small population which does not conform to this regular cycle.

(9) *Pegomyia hyoscyami* var. *betae* (mangold fly).

This was studied on the Wolds in the north of Lincolnshire. Egg counts in May would seem to be the ideal method of estimating the numbers of this insect, but unfortunately they do not always seem to be laid on the seedlings. There are two or three overlapping generations which render larval counts uncertain of interpretation. An estimate of the numbers of this species have been obtained by the percentage loss of leaf surface in September.

Experiments were made at the same time to find the loss of crop resulting from their attacks:

A 50 per cent. defoliation at the end of May causes 21 per cent. loss of crop.

A 50 per cent. defoliation in mid-September causes 5 per cent. loss of crop.

The percentage of leaf surface lost was:

1925	... 0·1 %	1928	... 3 %	1931	... 8 %	1934	... 2·5 %
1926	... 6	1929	... 50	1932	... 7·5	1935	... 0
1927	... 3	1930	... 50	1933	... 4		

It will be seen that 1929 and 1930 were the only years when this was a pest and even then only a very mild one. 1923 and 1924 were also slight pest years but no actual figures are available, only general notes.

REVIEWS

Insect Pests of Glasshouse Crops. By HERBERT W. MILES and MARY MILES. Pp. 174, 86 figs. in 21 plates, 15 text-figs. (Published by H. C. Long, "The Birkins", Orchard Road, Hook, Surbiton, Surrey.) October 1935. Price 8*s*. 6*d*. net.

The growth of glasshouse horticulture in this country has been rapid during recent years, and the cultivation of decorative plants and vegetables in glasshouses has become more intensive. The interest taken in the cultivation of plants under glass is in no way confined to the market grower for there is a vast company of private gardeners who take an intelligent and active interest in the cultivation of flowers, fruits and vegetables in greenhouses and conservatories.

The dearth of authoritative treatises dealing with the pests of horticultural plants is to be deplored, and it is fortunate, therefore, that the urgent need of a text-book dealing with the insect and allied pests of at least one group of these plants has now been realised and that the production of such a work has been in the hands of two well-known entomologists. The authors have gathered together in a moderately sized volume all the available information concerning the pests of glasshouse plants.

Mr J. C. F. Fryer, Director of the Ministry of Agriculture's Pathological Laboratory, in the Foreword states that "The factors of greatest importance in determining both the geographical distribution of insect pests and their abundance are climatic. It is, therefore, natural that in glasshouse cultivation, which is in effect the growing of plants in an artificial climate, the grower has to cope with a range of insect pests that differ in many ways from those found out of doors." The pests of glasshouse plants fall into two categories, the exotic species of insects which are associated primarily with tropical and subtropical plants and indigenous species which are constantly introduced—both directly and indirectly—into the houses, and there find conditions which are favourable to their rapid multiplication and development. The authors consider that the former group, with certain exceptions, are of less importance than the latter. While this statement may be true so far as commercial houses are concerned, it is not entirely applicable to the private grower in whose conservatory and greenhouse there is frequently little or no interval between crops.

The authors in their Preface express the hope that they have provided growers with an account of pests such as occur in glasshouses, and of the measures to be adopted for controlling the several parasites which exist under the special conditions of glasshouse horticulture. A careful survey of their efforts will show that their hopes are realised and that they have supplied the intelligent plantsman with a reliable treatise on the pests of glasshouse plants.

There are nine chapters, of which the first deals with Glasshouse Conditions in Relation to the Occurrence and Control of Pests arranged under sectional headings, namely the effects of glasshouse conditions; plant health and pest injury; glasshouse hygiene; and the nature of pests in glasshouses.

The subject-matter contained in Chapter I is of the highest importance to growers, many of whom require some practical guidance in the principles of hygiene. Some of the sins of omission of the owner of small greenhouses and conservatories require consideration in the section dealing with glasshouse hygiene in relation to infestation by pests.

Chapters II–VIII are devoted to a review of the more important insect and allied pests of glasshouse plants, namely eelworms, slugs, woodlice, millepedes and spider mites. Each species is considered in relation to its biology and habits, and the methods, both preventive and curative, are fully described.

The final chapter (Chapter IX) deals with the methods of controlling pests in glasshouses. The measures of the steam and chemical sterilisation of soils are described, together with the fumigation of glasshouses and the effect of humidity in relation to this operation. Finally, the several insecticides and the types of apparatus for applying both wet sprays and dry dusts are considered.

Appendix I provides a key to the pests associated with the chief glasshouse plants, which are arranged in alphabetical order. While the effect of the presence of pests on plants is apparent to the most unobservant plantsman, the provision of such a key is desirable even to the average grower who is not familiar with the stages through which insects undergo, and who must perforce depend on the signs of attack to determine the organism responsible for the injury.

Appendix II is a selected bibliography arranged under headings, *e.g.* Aphides, Capsids, Eelworms etc., Insecticides, Virus Diseases, Weed Suppression, and so on.

The Index is refreshingly complete for a book of this size, and should serve as a guide to authors of the advantages of introducing a complete guide to the subject matter in the text.

The numerous photographic illustrations are exceptionally good and all of them, with two exceptions, were specially taken by the authors for use in this book. A few of the text-figures are, on the other hand, of little merit, and the figure of the wingless viviparous female of *Myzus persicae* (p. 63) is valueless both to the scientific and the lay reader.

Proof-reading has been carried out with care, for the number of errors is small. One notes, however, the substitution of *Chorozema* for *Chorizema* (pp. 51, 164); *Irisine* for *Iresine* (pp. 81, 168); *Lecanium persicae* for *L. corni* (pp. 91, 156, 168); and the figures (72 and 74) on Plate XVIII, which should be transposed. The illustration of the earwig figured on Plate XIX is that of the var. *forcipata*.

It is stated by the authors that the list of injurious species is inevitably incomplete, but the number of omissions is small and in no way detracts from the value of the work.

The more apparent omissions are those dealing with general information. For instance, no mention is made of the following facts: the treatment of loam heaps with carbon bisulphide against wireworms (p. 28); the susceptibility of tuberous-rooted begonias to attack by the larva of the vine weevil (p. 29); the frequent occurrence of *Aphodius* larvae in glasshouse soils into which they are unwittingly introduced with manure (pp. 30–1); the employment of nicotine dusts for controlling *Tortrix* leaf-tyers (p. 55); the relation of weed hosts to the chrysanthemum leaf-mining fly (p. 57); the relationship between *Aphis gossypii* and lily mosaic (p. 71); the acknowledgment that the late Maxwell Lefroy was the pioneer in the work of introducing the Chalcid *Encarsia* as a biological agent in controlling the greenhouse white fly (pp. 83–4); the danger attending the application of white oil emulsions to the foliage of certain palms (p. 96); confirmation of the statement that *Thrips tabaci* is a proved vector of yellow stripe of narcissus (p. 98); the discoloration of tomato foliage as a result of an attack of woodlice at the roots (pp. 123, 157); the conditions favourable to Collembolids in greenhouses and conservatories (pp. 125–6); the efficacy of Japanese ant bait in controlling ants in frames, propagating pits and glasshouses (p. 130); the mention that *Potash* soft soap is necessary as a wetter (p. 149); the injury to the foliage of palms by young slugs (p. 156); and the omission of the species of Tarsonemid mites which are primary pests of begonia, cyclamen, fuchsia and other subjects.

The inclusion of a number of cross-references is desirable, for it must be realised by authors that some readers consult a work for the sole reason of obtaining information on a particular subject and thereby fail to obtain the fullest information available in that work. For instance, the list of plants which are intolerant of tetrachlorethane vapour mentioned on p. 144 should be referred to on p. 85 when the chemical is discussed in relation to white fly control.

The authors deserve the highest praise for the production of a text-book which will rank for some years as an authoritative treatise on glasshouse pests. The format of the book is good, though some readers will not be in sympathy with the introduction of advertising matter. The price is reasonable, and this book should find its place on the shelves of every intelligent plantsman for it will prove to be invaluable both to the market grower, the private gardener, the student, and to every worker in the field of applied biology.

G. FOX-WILSON.

The Living Garden, or the How and Why of Garden Life. By E. J. SALISBURY, D.Sc., F.R.S. Pp. xi+338, 63 figs. and 17 plates. London: G. Bell and Sons, Ltd. 1935. 10*s.* 6*d.* net.

The increasing application of science to horticulture is a striking fact of modern times. The sharp cleavage between the pure botanist and the practical gardener and grower is rapidly becoming narrower. There have, of course, always been botanists who were also keen horticulturists, but until recent years there have been fewer physiologists who combined their knowledge of the science of plant behaviour with an understanding of the art of garden practice. The gain to both by the combination has never been more clearly demonstrated than in Prof. Salisbury's new book. He does for horticultural physiology what so many writers have done for other sciences. He collates from a hundred sources the accumulation of knowledge on the physiology and botany of garden plants, synthesises it with his own wide knowledge of botany and garden art as a catalyst, and presents it in a form which is delightful to read and stimulating to ponder upon. The happy blend of garden lore with botanical and physiological knowledge will be a joy to all plant and garden lovers.

His choice of subjects ranges over the whole field of botany and horticulture, from recent work on photoperiodism, vernalisation, and the physiology of propagation, to the ways in which plants have obtained their names and the old time "floral clocks" of Loudon.

Many attempts to popularize science fail because the writer tries to describe mechanisms rather than the effects of mechanisms. For example, in nearly every book on popular biology, the chromosome basis of heredity is explained "in clear and simple language", to the detriment of the book's interest. Prof. Salisbury does not fall into this trap; the effect of the mechanisms are described and the result is a far clearer picture to the non-scientific reader. But the book is one which will be read with pleasure and profit not only by the horticulturist but by the botanist as well, for much of the information which is gathered together is outside his usual purview, and yet may have a bearing on his "pure science".

Errors and misprints are few, but it is perhaps surprising to note the departure from the Rules of Botanical Nomenclature in the case of names of hybrids, by the placing of the sign of hybridity before the generic name instead of between this and the specific or varietal designation. Ribston Pippin apple, named after Ribston Hall in Yorkshire, is consistently misspelled "Ribstone", and on p. 179 "Pearmain" is wrongly given as "Permain". On p. 180 the unpronounceable *Pyrus Niedzwetzkyana* is spelt *P. Niedswetkyana*. The reference on p. 14 to the dwarfing stocks for apples is obviously meant to refer to East Malling Stock No. IX, and not to the little-used and unsatisfactory No. IV. It would be interesting to know the evidence on which the statement is made that the cherry variety, Hertfordshire Black, is an exception to the otherwise invariable rule for practical purposes of self-incompatibility in the sweet cherries. If sound, geneticists will be faced with a new problem to explain, and presented with a possibly valuable parent for breeding experiments.

The pen and ink illustrations by Mrs Caroe, many of them in the style of the old herbals, but lacking the inaccuracies of these, are not the least pleasant features of the book. It is rarely indeed that a book on any aspect of plant biology finds its way into the eclectic ranks of the "best sellers"; the fame of this book went far and wide even before its publication, and it will find a place on the shelf of all those who, knowing the "how" of horticultural practice, are interested also in the "why".

R. H. STOUGHTON.

Our Enemy the Termite. By T. E. Snyder. Pp. xii + 196, with 10 plates and 56 text-figures. Ithaca. New York: Comstock Publishing Co. 1935. $3.0.

The author of this manual is Senior Entomologist under the United States Department of Agriculture. Since 1909 he has been chiefly occupied (in his official capacity) in investigating the biology, and means of control, of termites and other wood-destroying insects. Much of the first-hand knowledge thus acquired forms the basis of this useful and very readable book. It is, in fact, an authoritative introduction to its subject, being both accurate and up-to-date. While its method of treatment is essentially scientific, its clarity of style and freedom from undue technicality should make it acceptable to the layman. Any unfamiliarity with certain of the words used need not cause perplexity, for an adequate glossary helps those not versed in the particular terminology. The book treats of the American species only, but this limitation is less pronounced than may appear at first sight, since most aspects of termite life come in for discussion. The first six chapters are concerned with termite ancestry and affinities: metamorphosis and castes: flight and reproductive behaviour: habitations or nests: nutrition and inquilines. These take the reader to p. 98, while the remainder of the book is concerned with termites in relation to man. It has been estimated that $40,000,000 damage is caused annually by these insects in the United States, so that their control becomes a matter of real importance. The injuries which they entail to buildings, crops, etc., and how they are to be avoided, or made good, are dealt with at some length. The problem of control is far from simple and success depends upon adequate ecological knowledge of the individual species concerned. Dr Snyder has much to say about the principles of termite-proof building construction and the conservation of structures already infested. He also provides building codes whose axioms he recommends to the householder and contractor. There is little doubt that, if regular and systematic attention be paid to certain essential principles of building construction, less would be heard about the termite menace than in the past. The general reader will find most of what he may want to know in this manual, while the biologist will be sufficiently interested in it to wish for greater detail and a bibliography. The many illustrations are good and the index serviceable.

A. D. IMMS.

Soils? their Origin, Constitution and Classification (An introduction to Pedology). By G. W. Robinson, M.A. 2nd Edition. Pp. 442 + xvii, with 5 plates and 17 figures in text. Thos. Murby and Co., 1 Fleet Lane, London, E.C. 4. 1936. 20*s*. net.

The first edition of this work appeared in 1932, and its recognition by teachers and research workers all over the world as an authoritative text-book of outstanding merit on soil science was immediate. Most text-books treat the soil more or less from a purely utilitarian point of view, but of recent years soil science has emerged from a position intermediate between the pure and applied sciences into a science known as Pedology, in which the soil is studied from the standpoint and by the methods of pure science. This was the first text-book in English to treat the subject "philosophically", its primary object being for those "interested in the soil as an object of study in itself, and secondarily for those whose interest lies in its economic or geographical significance". "The standpoint adopted is the exhibition of soils in their natural relationships."

That a second edition should follow in so comparatively short a time is the result of the author's desire to amend, clarify and augment, and bring right up-to-date much of the original material in view of the universal approval which greeted the first edition, and the very large quantity of new data published during the interval together with the emergence of many new ideas on various aspects of the subject. The narrative, after allowing for excisions and replacements, has been increased by 63 pages. Several plates, diagrams, and bibliographical references have been added, and of the latter there are some 500.

The second edition follows the same general lines as its predecessor. The general aims of Pedology are outlined in the introductory chapter, and the remainder of the book may be regarded as falling into three sections. The first deals with the origin, constitution and properties of soils, including general physical properties, water and air relationships and the role played by organic matter and soil micro-organisms. This section is excellently written, and the enormous complicated mass of material is dealt with in a very concise and lucid manner. The next section is concerned with soil genetics; the chief soil groups of the world and the difficult problems of classification are discussed at some length. A unique and valuable feature of the book is the description of concrete examples of soils from different parts of the world affording a useful source of reference to types likely to be found in widely separated countries. The final section deals with methods of soil survey and classification into series, soil analysis, and plant growth and agriculture. The soil profile, as seen in the field, is the proper unit of study, and laboratory analyses are useful for the confirmation of field observations and the elucidation of problems associated with the mode of soil formation, etc., as well as providing quantitative expressions of certain soil properties. The large amount of original work carried out by the author himself in these directions is well known.

To ecologists, botanists, and plant physiologists a wide knowledge of the properties of soils in their various stages of evolution is invaluable, and in this treatise they will find a comprehensive, detailed and systematic treatment of the subject. The book is very well written indeed, and Prof. Robinson is the happy possessor of a literary style that is always pleasant, interesting and fresh.

M. N. NICHOLSON.

REPORT OF THE COUNCIL OF THE ASSOCIATION OF APPLIED BIOLOGISTS FOR THE YEAR 1935

THE Association has met on six occasions during the year, including one Field Meeting and one afternoon Excursion. The Annual Summer Meeting was held on July 5 at the East Malling Research Station by kind permission of the Director, Mr R. G. Hatton, and the Excursion was to the Lister Institute of Preventive Medicine on December 13 by kind permission of the Director, Prof. J. C. Ledingham. To both these Institutions the Association is indebted for their hospitality.

The attendance at ordinary meetings has been on the average, 37 Members and 21 Visitors.

Twenty-two Ordinary Members were elected during the year, including three old Members who rejoined. Three Members resigned and the Association now numbers 308 Members including 12 Honorary Members. Of the Ordinary Members 242 are resident in the British Isles and 54 in the Empire and foreign countries.

During the past year the Association has again enjoyed the privilege of holding its meetings in the Botanical Department of the Imperial College of Science and Technology and in the Metallurgical Lecture Theatre of the Royal School of Mines, and the Council takes this opportunity of recording its grateful thanks, on behalf of the Association, to the College Authorities for this valued hospitality.

The following papers and discussions were brought before the Association during the year 1935.

Feb. 15. (1) Mr D. WARD CUTLER and Miss L. M. CRUMP: "Nitrification by Micro-organisms other than Nitrosomonas."
(2) Dr A. S. CORBET: "Recent Work on the Biological and Chemical Oxidation of Ammonia to Nitric Acid."
(3) Mr W. G. E. EGGLETON: "The Oxidation of Ammonia in Soil Incubation Studies."

Mar. 22. (1) Dr H. W. MILES: "Sawflies associated with Cultivated Rosaceous Plants."
(2) Mr R. B. BENSON: "The Alien Element in the British Sawfly Fauna."

Oct. 11. (1) Dr W. F. BEWLEY: "Some Glasshouse Problems at the Cheshunt Experimental Station: Introduction."
(2) Mr E. R. SPEYER: "Animal Pests of Interest in Glasshouses."
(3) Dr O. OWEN: "Soil Problems in Glasshouses."
(4) Mr W. H. READ: "The Control of the Red Spider Mite and Tomato Leaf Mould."
(5) Mr P. H. WILLIAMS: "Some Important Diseases of Glasshouse Plants."

Nov. 15. (1) Dr H. F. BARNES: "Insect Population Studies in the Gall Midges."
(2) Mr A. ROEBUCK: "Field Observations."

G. FOX-WILSON
R. H. STOUGHTON } *Hon. Secretaries.*

REPORT OF THE HON. TREASURER FOR THE YEAR ENDING DECEMBER 31, 1935

DURING the year ending December 31, 1935, subscriptions and entrance fees (including arrears paid up) received from members amounted to £316. 16*s*. 6*d*. This is an increase of over £30 from last year, but is less than the amount received in 1931 by £20. Income from the sales of the current volume of the *Annals of Applied Biology*, and from reprints amounts to £690. 1*s*. 0*d*., an increase over last year of £78. 0*s*. 9*d*. The size of the volume of the *Annals*, which had fallen in 1934 to 728 pages from 792 pages in 1933, was raised to 820 pages in 1935 (including 11 pages of the List of Members); and the cost of producing it has risen by £173. 13*s*. 11*d*. to £1106. 14*s*. 5*d*.

Over the whole year there has been an excess of expenditure over income of £30. 8*s*. 9*d*., approximately the same as for the last two years, the increased expenditure being balanced by increased income. After all obligations have been met, the assets of the Association amount to £1054. 7*s*. 9*d*., of which £731. 5*s*. 0*d*. is represented by National Savings Certificates.

The financial position of the Association is therefore satisfactory at the present time. It is important, however, that every opportunity should be taken of enlarging our membership, and it is very desirable that members keep up to date the payment of their subscriptions.

J. HENDERSON SMITH,
Hon. Treasurer.

THE ASSOCIATION OF APPLIED BIOLOGISTS

Dr. *ANNALS OF APPLIED BIOLOGY* INCOME AND EXPENDITURE ACCOUNT FOR THE YEAR ENDED DECEMBER 31, 1935 *Cr.*

EXPENDITURE	£ s. d.	£ s. d.
To Estimated Value of Stock at January 1, 1935		85 18 6
To Cambridge University Press		1106 14 5
To copies bought in		10 13 3
		£1203 6 2

INCOME	£ s. d.	£ s. d.
By Sales—Current Volume		549 0 0
By Sales—Back Volumes, Parts and Sets		62 6 9
By Sales of Reprints		141 1 0
By Advertisements		3 15 5
By Estimated Value of Stock at December 31, 1935		108 12 6
By Balance carried down		338 10 6
		£1203 6 2

Dr. GENERAL INCOME AND EXPENDITURE ACCOUNT FOR THE YEAR ENDED DECEMBER 31, 1935 *Cr.*

EXPENDITURE	£ s. d.	£ s. d.
To *Annals of Applied Biology*, balance brought down		338 10 6
To Printing and Stationery		6 6 8
To Postages and Cheque Stamps		7 7 2
To Honorariums		9 15 0
To Sundry Out-of-Pocket Expenses of Secretaries and Treasurer		7 17 0
To Audit Fee Reserve		4 4 0
		£374 0 4

INCOME	£ s. d.	£ s. d.
By Members' Subscriptions:		
Arrears	22 10 0	
Entrance Fees	6 16 6	
Current	287 10 0	
		316 16 6
By Interest on National Savings Certificates and Bank Deposit		26 15 1
By Balance, being Excess of Expenditure over Income for the year		30 8 9
		£374 0 4

BALANCE SHEET, DECEMBER 31, 1935

LIABILITIES AND SURPLUS	£ s. d.	£ s. d.
Sundry Creditors:		
Cambridge University Press	360 18 0	
Audit Fee Reserve	4 4 0	
Sundry Expenses	11 10 8	
		376 12 8
Subscriptions and Entrance Fees paid in advance		14 14 5
Excess of Assets over Liabilities:		
As Balance Sheet, of December 31, 1934	1084 16 6	
Less: Balance of Income and Expenditure Account for 1935	30 8 9	
		1054 7 9
		£1445 14 10

ASSETS	£ s. d.	£ s. d.
Cash:		
At Bank on Current Account	223 7 4	
At Bank on Deposit Account	350 0 0	
		573 7 4
Debtors for Subscriptions two years or less in arrear and considered good		32 10 0
500 National Savings Certificates		731 5 0
Stock of *Annals of Applied Biology* at estimated value		108 12 6
		£1445 14 10

J. HENDERSON SMITH, *Hon. Treasurer.*

We certify that the foregoing Accounts are properly drawn up in accordance with the books, vouchers and documents produced to us, and, in our opinion, the Balance Sheet exhibits a true and correct view of the state of the affairs of the Association.

(Signed)
H. J. COX & CO.
Incorporated Accountants
Auditors.

HARPENDEN, *February* 10, 1936.

S. P. WILTSHIRE.

Vol. XXIII, No. 3

August, 1936

THE ANNALS OF APPLIED BIOLOGY

EDITED FOR THE ASSOCIATION OF APPLIED BIOLOGISTS

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AND

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ERRATA

Pages 231 and 244, *for* Warrington *read* Warington

Page 320. Legend of Text-figure 5, *for* Fig. 4 *read* Fig. 5

Plate XVI. Reverse indicator lettering for Fig. 2

Page 448. Review of Robinson's *Soils*, delete "?" in title

VOLUME XXIII AUGUST, 1936 No. 3

ZONE LINES IN PLANT TISSUES

III. THE BLACK LINES FORMED BY *POLYPORUS SQUAMOSUS* (HUDS.) FR.

BY A. H. CAMPBELL, B.SC., PH.D.
AND R. G. MUNSON, B.SC.
From the Department of Botany, University of Bristol

(With Plates XVIII and XIX)

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I. INTRODUCTION

POLYPORUS SQUAMOSUS (HUDS.) FR., the great scaly *Polyporus*, is well known in Europe as a tree-rotting fungus. Its sporophores have been recorded on a wide range of dicotyledonous trees, including apple, pear, mulberry, horse-chestnut, lime, basswood (*Tilia*), willow, ash, oak, walnut, birch, beech, maple, and elm. Von Tubeuf & Smith (19) include living hazel and mountain ash. Carleton Rea (17) adds yew, but, since no other record has been found of the occurrence of this fungus on a conifer, it must be regarded as very exceptional.

The fungus is a wound parasite with apparently little power directly to affect the living parts of the wood. Thus the attacked tree generally shows extensive heart rot and dies slowly. The decay of the heartwood is usually far advanced before the fungus spreads to the more peripheral parts of the wood. In the later stages of the rot the wood is broken up by mycelial plates and eventually the complete breakdown of the tissues results in the characteristically hollow centre. It is usual, therefore, for several years to elapse after infection before the typical bracket sporophores are produced. These may arise singly or in clusters, the base of the stipe being typically black in colour. The development of the sporophore occurs rapidly during the summer and early autumn, the full size, 14–18 in. in diameter, being attained in 2–3 weeks' growth. It lasts only a single season and the breakdown generally begins in the month of November.

The literature dealing with *Polyporus squamosus* centres mainly around the development of the sporophore and the wood decay induced by the fungus. Buller(5) has given an account of the biology, while Brooks(4) has confirmed a number of his observations on spore discharge and contributed further data on the rapid growth of the sporophore. In 1895 Cooke(9), after mentioning that *Armillaria mellea* is credited with being the complete development of one of the species of *Rhizomorpha*, states that another form or variety of *Rhizomorpha* is undoubtedly the mycelium or vegetative condition of *Polyporus squamosus*. More recently Ramsbottom(16) states that wood affected by *P. squamosus* shows a black line similar to that caused by *Armillaria mellea*. Mention is made by Buller(5) of the black layer of mycelial tissue, which is frequently to be found in or upon wood rotted by *Polyporus squamosus* and other Polypori, and a description is promised in a later paper. To our knowledge no further account has appeared; this investigation is directed at the neglected portion of the biology of *P. squamosus*, the formation of "black lines".

One of us (A. H. C.) had collected at Edinburgh in 1931 a group of sporophores of *P. squamosus* arising from the trunk of *Ulmus campestre*, the wood of which showed obvious black lines beneath the point of origin of the sporophores. A search was made for fresh material with which to start this investigation; but considerable difficulty was experienced in finding the fungus in the summer of 1934. The absence of fructifications of what is, typically, a common fungus was attributed to the hot and dry summer of that year. In this connexion, it is interesting to note that Duggar(10), referring to conditions in North America, states that *Polyporus squamosus* appears to be uncommon in regions which are rather dry throughout the summer. Eventually, two rather spent sporophores of *P. squamosus* were found on a fallen elm at Yate, near Bristol. Associated with these sporophores were a number of black lines in the wood of the trunk.

II. Development of the fungus in pure culture

The isolations of the fungus were carried out from wood in the neighbourhood of the black lines, a number of pieces being taken from both sides of the line. A substantial percentage of these pieces of wood, when transferred to malt-agar slants, yielded a fungus which, when freed from bacteria, was identified as *P. squamosus*. Unfortunately, the age of the sporophores prevented the isolation of the fungus from the basidiospores; so, in order to confirm the diagnosis, the wood isolations were compared with a culture of *P. squamosus* from the Forest Products Research Laboratory, Princes Risborough; they were found to be identical within the limits of cultural variation. In general, the Yate fungus appeared to be much more vigorous than the culture from Princes Risborough.[1]

Although the development of *P. squamosus* has previously been described by Buller (5) from gross cultures on wood, and by Price (15) from pure cultures on wood inoculated with spores, no satisfactory account exists of the development of the fungus in pure culture on artificial media. Thus, the facts presented here are intended only to supplement the existing accounts. Any complete description would involve so much restatement as to be unjustified.

Growth of the fungus occurred readily on a variety of artificial media such as malt extract, potato dextrose and oat-extract agars. On pure agar, mycelial growth is scanty and ceases early. These cultures are incubated at 25° C. unless otherwise stated. Typically, development takes the following course: on transferring a portion of the isolated mycelium to a fresh malt-agar slant, very little change is seen for the first 2 days. About the third day, hyphae grow out from the inoculum as long hyaline threads 2–3 μ in diameter with few branches. These form a loose cottony mycelium which spreads over the agar, the advancing margins consisting of unbranched hyphae running along the surface of the agar but not appressed to it. These continue to advance for the next 3–4 days at the rate of about 1–1·5 mm. per day. At 3–5 mm. behind the foremost hyphae the branching is considerable, forming a loose felt where colourless drops, which disappear later, are seen. Then, at the sides of the agar slants, a marked aggregation of hyphae is seen, and from this plexus arise groups of short papillae. These are usually found in rows between the agar and the glass, generally so pressed against the glass as to cause a noticeable indentation of the agar. The papillae are formed as whitish yellow, pseudoparenchymatous masses 0·3–0·6 mm. in diameter and 0·5–1·5 mm. in height but varying considerably in size, according to the medium upon which they are formed. On the Princes Risborough fungus, especially, they seemed to take the form of a short tube which became more distinct on the drying out of the culture.

---

[1] Our thanks are due to Mr K. St G. Cartwright who sent us this culture of *P. squamosus*.

Frequently, when massed together, they give the impression of a number of isolated pores from a polypore fructification. These papillae, to which no function can as yet be ascribed, are formed both in the dark and in the light; they show apparently no geotropic response, since they are formed, irrespective of any outside influence, against the wall of the culture vessel.

At the period when papillae are forming, the hyphae show numerous clamp connexions and, shortly afterwards, brown patches appear either on, or just below, the surface of the mycelium. These patches increase in size and, where they touch the wall of the tube, appear as a thin black line. This is usually to be observed about the fifth or sixth week and is more conspicuous in potato-dextrose agar slants than in malt-extract or oat-extract agars.

Where mycelial growth has been vigorous, resulting in the filling of the lower part of the tube with vegetative mycelium, the hyphae, towards the mouth, come to form a more or less flattened surface, which, becoming discoloured and more closely interwoven, forms a thin brown plate over the rest of the hyaline mycelium (Pl. XIX, fig. 1). This plate is very brittle and, where it intersects the wall of the tube, shows as a line which, when the fungus is growing on oat-extract agar is brownish but, with malt-extract and potato-dextrose agars, is more black. As desiccation proceeds, the loose cottony mycelium below this line contracts and eventually withers; but the plate remains *in situ*, frequently forming oidia on the upper surface.

Where the mycelial growth has been less vigorous, the light cottony mycelium collapses slightly: the initiation of line formation begins, usually near the inoculum, by the darkening of the hyphae. This darkening then spreads, sometimes beneath the surface of the mycelium and thus not easily visible, until it shows as the dark line at the glass.

Frequently, the plate is not completed over the mycelium. Instead, the mycelium beneath, apparently due to some protection afforded by the plate, is enabled to maintain or to recommence active growth. Hyphae grow round the plate and frequently through it, especially on potato-dextrose agar, until the original plate may be completely covered. This secondary growth then proceeds normally to form a second plate over the new growth, as can be seen in Pl. XIX, fig. 1.

In yet further cases of the development of the fungus, most frequently on malt agar in conditions of relatively low humidity, only a thin layer of mycelium is formed which may show isolated dark patches but never reaches the plate stage. In such, the papillae become discoloured and dry out, the mycelium then breaking up into masses of oidia which present a dusty yellow appearance.

On "C" medium (7) mycelial growth is vigorous, resulting in the course of 8–10 weeks in the thorough permeation of the medium by a dense weft of hyaline hyphae. In all cultures on this medium there is eventually formed, on the surface of the mycelium, a thick dark plate whose characteristically dusty appearance is due to the presence of large numbers of oidia. On one such culture on "C" medium, in a litre conical flask, there appeared, 5 weeks after inoculation with the Yate isolation, a white hemispherical mass growing out from beneath the dark plate and oidial layer. The mass was closely pressed to the glass and it apparently arose from an aggregation of white mycelium at the side of the flask (Pl. XIX, fig. 3). The white mycelium was bounded by a thick black layer about 2 mm. in diameter, the whole arrangement strongly recalling the pseudosclerotia of *Armillaria mellea* as they have been described buried in the culture medium (8). During the 4 days following its appearance, the mass further developed as a double body of a definite bracket shape. Thereafter, growth appeared to be finished and, in an attempt to stimulate the production of the pores and basidiospores, the culture was removed to another incubator at the same temperature, i.e. 25° C., but where it was exposed to full daylight. This was on 21 June. No further growth of this bracket-shaped body occurred, but there appeared arising from it, first one and then successively three other white processes, all in the space of 3 days. These structures were curious, each finally being from 5 to 8 cm. in length and consisting of a much swollen base and a slightly flattened apex, with an indefinite number of finger-like projections. Later, darkening in colour, they branched frequently, so that, a fortnight after their appearance, they had almost filled the flask. The culture was next removed from the light incubator at 25° C., to room temperature, with the result that on the following morning the entire growth had collapsed, liberating large quantities of water. No further development was seen in the flask.

In 4 months old cultures on malt agar, grown at room temperature in the light, a number of club-shaped bodies, similar to those described by Price (15) as occurring on wood blocks, have been observed. These are shown in Pl. XIX, fig. 2, and correspond to the structures developed on "C" medium. Actually they are much smaller, generally less than 4 cm. in length, and are cylindrical, terminating in a small flattened cap, suggestive of an attempt to form a pileus. No pore formation, however, has been seen on the structures developed on either "C" medium or malt agar. At the surface of these bodies there is a simple palisade layer of hyphae on which oidia are formed in large numbers.

Price (15) has described these club-shaped bodies as abnormal fructifications, and this investigation endorses that view. Similar sterile structures formed by *Polyporus squamosus*, when growing on naturally infected wood in the dark, have been described by Buller (5), who further states that pileus production is brought about entirely by the morphogenic stimulus of light. If this is the case, it is difficult to explain why the attempted fructifications, developed in the cultures in the light, failed to produce pilei. It is reasonable to conclude that other factors than light are concerned in pileus formation, but these must necessarily remain undetermined until typical sporophores are produced in artificial culture.

An account of the development of *P. squamosus* on wood blocks has been given by Price (15), who obtained cultures by sowing spores on the surfaces of sterilized wood blocks. Two months were required to produce an obvious mycelium at room temperature, and mycelial transfers required 1 month for the formation of a thin layer of mycelium. After 3–4 months a dense white felt covered the whole surface. This felt then became pale brown in colour and produced oidia. On cultures 5–10 months old what are described as abnormal fructifications developed.

The present observations confirm these statements. Sterilized wood blocks of poplar, sycamore, pear, elm, beech, ash and oak were inoculated by mycelial transfer. A few days after inoculation, the typical loosely branched hyphae of the fungus form a small tuft of mycelium which gradually spreads to form a thin layer over the surface of the block. In about 6 weeks the block is enclosed in a thin weft of mycelium which now shows a more active external development, the growth having been previously directed towards penetration. The hyphae become more closely interwoven, especially at the top of the block, to form a sheathing plate of "black line" over the upper part of the block, similar to the "line" formed in agar-media cultures. Formation of the "black line" extends over the block and may include the cotton-wool below the block, as has been described (7) for *Xylaria polymorpha*. Drops of dark-coloured liquid, apparently excreted from the mycelium, are to be seen at this time.

With cultures on malt-agar slants, to which a wood block is later introduced, the introduction of the wood block is followed by a period of intense vegetative activity. Loose cottony mycelium quickly grows over the block and, in 8–10 days, completely immerses it, so permitting a very rapid invasion of all sides of the block. The fungus appears to attack the block very readily while it is still moist, but, if drying out should occur at the upper end before the mycelium reaches it, the development is much slower. On one such block, where the fungus had been able to attack only the lower end, black lines were found on removing the mycelial mat. The age of the culture was only 5 weeks. Sections of the wood showed that the lines so formed did not extend more than 0·1–0·2 mm. into the wood, and in one case the line was found to mark the limit reached by the fungus.

Oidia have appeared on all cultures, other than those on pure agar, where the mycelium was never properly established. They appear to be formed as the result of a check to the vegetative growth of the mycelium and their function is obscure. The oidia are produced by the fragmentation of the hyphae into chains of single cells, 8–30μ in length and 4–10μ in diameter. They are of irregular form but, when mature, are typically ovoid, hyaline, with thick walls and densely granular protoplasm. The cell wall usually shows a small protuberance at one end of the spore. In fixed and stained oidia, two large vacuoles are commonly present, situated at each end of the cell, while between them appear two darkly stained structures which correspond, in size and appearance, with the nuclei of the hyphae. Germination of the oidia failed to take place in sterile tap water over a period of 14 days; but cultures, undoubtedly derived from oidia, have been obtained on malt agar. Unfortunately, the germination seems to be extremely sporadic, and consequently the process has not been observed.

III. DEVELOPMENT OF THE BLACK LINE

In cultures on artificial media, more especially on malt-extract agar, in test-tubes, boiling tubes and Petri dishes, the hyphae submerged in the medium become aggregated into the position of the subsequent black line. Here they first form a colourless plate passing down through the agar, continuous with the dark plate on the surface of the medium. Darkening of this colourless plate then proceeds from the surface down into the medium, until the plate ultimately ends at the glass wall of the culture dish.

Microtome sections of the initial stages of plate formation show a palisade structure of closely packed hyphae. This palisade is the result of the close branching of a number of hyphae, all of which are growing in the same direction. Thus, once the palisade is completed, appearing as a black line, it becomes obvious that the hyphae forming it have all arisen from a single side of the line: hyphae are plentiful on the other side, but take no part in the formation of the palisade. Pl. XVIII, fig. 5, shows this formation in progress. Later, the hyphae lose their cell contents, have thicker walls and become bladder-shaped. So far, the only perceptible difference between the ordinary hyphae and the bladder hyphae is in the swollen cells of the latter, since both are stained by the usual cellulose stains. Soon after this stage, yellow colouring matter appears on the walls of the bladder hyphae, producing definite opacity in the swollen cells. Corresponding with this change of colour, a change in the constitution of the cell walls is noticeable, in that they no longer stain with the common stains. This change is identical with the sclerotization of the cells forming the rind in the sclerotium of, for instance, *Polyporus umbellatus* (3).

Examination of sections of the line, produced by *P. squamosus* when growing on ash blocks in pure culture, show that the "line" formed in the wood has a structure directly comparable to the plate formed in the artificial media. Generally, sections through the "lines" formed in inoculated wood blocks show the "line" to be at a later stage in development than can be seen in artificial media, in that the walls of the bladder cells are now stained dark brown in colour.

Again, examination of the black line present in the wood of the Edinburgh and Yate material of *P. squamosus* revealed a structure identical with that of the line produced in the inoculated blocks. The bladder cells composing the tightly packed line, however, are now darker, being mostly black in colour, so that only in a thin section can the composition of the black line be seen.

The dark impregnating substance found on the bladder cells is very refractive and very little is known of its composition and formation. Its organic nature is shown by the fact that it can be bleached by Eau de Javelle, but it resists acids and alkalis and is insoluble in all the usual organic solvents.

IV. SIGNIFICANCE OF THE BLACK LINE

The black line in the wood attacked by *P. squamosus* is really a section through a dark plate, composed of tightly packed bladder hyphae and the remains of the wood elements in which they were formed. A remarkable feature of this black plate is its hardness and the fact that it is able to preserve its shape and entity, even when the surrounding wood has rotted away. From an examination of such rotted specimens, the black plate is seen to trace out in the wood the periphery of a large irregular body, sunk some distance in the wood. Part of the periphery of the body is generally to be found, however, in the bark of the tree or between bark and wood, and, at this point, direct connexion between the sporophores of the fungus and the body in the wood is evident.

One of us has already described a similar black line, formed by *Armillaria mellea*, in the wood of attacked plants (8). In this publication is described how the black plate forms the bounding layer, or rind, of a pseudosclerotium partly buried in the tissues of the host. The rhizomorphs beneath the bark (*Rhizomorpha subcorticalis*) and in the soil (*R. subterranea*) are regarded as extensions of the pseudosclerotium, and upon them are borne the sporophores. In the case of *Polyporus squamosus*, the pseudosclerotium, for such it is, bounded by the black plate is almost wholly immersed in the attacked wood, the sporophores apparently arising directly from the bark of the tree. It should be noted in this connexion, however, that Bolton has illustrated, in the British Museum collection of drawings, an antler form of *P. squamosus* growing from a dark brown sclerotioid base (18).

V. SCLEROTIA IN THE GENUS *POLYPORUS*

A survey of the described sclerotia in the genus *Polyporus* indicates how widespread are these productions. Gaumann (13) lists *P. tuberaster* in the north temperate zone, *P. Berkeleyi*, *P. umbellatus* and *P. frondosus* in the United States of America, *P. sacer* and *P. Goetzii* in Africa, *P. sapurema* in Brazil, *P. rhinocerotis* in the Malay region and *P. basilapidoides* and *P. Mylittae* in Australia as sclerotium-forming species. Some account of these sclerotia is of interest, since they are by no means homogeneous in structure.

According to Berkeley (2), the "fungus-stone" (Pietra fungaja) is constantly used in Italy for the propagation of *P. tuberaster*. He states that the "fungus-stone" consists of a ball of earth and stones, matted together by the mycelium of the fungus. Bommer (3) has given a more detailed account of the Pietra fungaja: he takes the view that the proportion of earth and stones in the ball has been much exaggerated and that it is in fact a true sclerotium. He points out that the presence of a definite black rind, differentiated from the hyaline hyphae of the pith, distinguishes this body from a mere aggregation of mycelium.

The large sclerotium of *P. Mylittae*, the "Blackfellow's Bread" of Australia, consists of a much more uniform mass of fungal plectenchyma. This sclerotium, which may attain a considerable size and 10–12 lb. in weight, represents a large reserve of carbohydrate material, mainly in the form of fungal cellulose, stored for the nourishment of the sporophores (11).

P. sapurema is also developed from a large sclerotium, which is leathery and furrowed, and may reach a diameter of 30 cm. (14)

Fischer (12) has described the sclerotium of *P. sacer*. Briefly, it consists of a hyaline pith, made up of several kinds of hyphae, and a thick brown rind of two distinct layers. According to this author, the sclerotium of *P. sacer* is very similar to Pachyma cocos but of smaller size. The sclerotium known as Pachyma cocos, although of fairly common occurrence, has not been observed to form sporophores and hence its identity is as yet unknown. Pachyma cocos is of great interest, since it is undoubtedly formed by a fungus growing in tree roots and, in consequence, a considerable proportion of the wood tissues of the root are to be found in the sclerotium (1, 12). Butler (6) mentions *P. sacer* developed from another form of sclerotium, the "tiger's milk" (Susu rimau) of the hill tracks of Assam and Malaya. This is a sclerotium of quite extraordinary hardness.

The sporophores of *P. umbellatus* are to be found arising from a large blackish sclerotium (*Sclerotium giganteum* Rostr.). Bommer (3) found a specimen of this sclerotium near Brussels, occupying a square metre in area and spreading over a depth of 40 cm. in the soil. The sclerotium is composed of large blackish branches, irregularly cylindrical or flattened and measuring 2–3 cm. in diameter, which are apparently connected with the roots of neighbouring oak and beech trees. A section through one of these roots shows that the whole of the interior is filled with mycelium and only the outer layers of the root remain as a cover to this part of the sclerotium. The mature sclerotium has a distinct rind, formed by the sclerotization of the walls of the superficial hyphae, which are eventually transformed into a mass of brown refractive substance. In the internal substance of the sclerotium is white in colour and reminiscent of cork in its consistency.

It would seem that the sclerotia in the genus *Polyporus* are distinguished from mere aggregations of mycelium, such as occur in *P. lucidus* and *P. tumulosis* (3), by the differentiation into rind and pith. The distinction is obviously based on rind formation and rightly so, as the thickening of the cell walls and the depositing of the brown refractive substance (sclerotization) is so characteristic as to be unmistakable. The formation and structure of the black line of *P. squamosus* in attacked wood is so closely similar to that of the rind of, for example, *P. umbellatus* that their homology is clearly established. The shape of the much-branched sclerotium of *P. umbellatus* suggests a further analogy between this body and the pseudosclerotium of *P. squamosus*. For the sake of simplicity, the black line has hitherto been described as a section of the rind of a pseudosclerotium, of more or less simple shape, such as is found in the case of relatively sound wood. Sometimes, however, in much-rotted wood, a much larger number of black lines are to be found producing an intricate design. This can be readily interpreted, if the shape of the sclerotium of *P. umbellatus*, with its large number of densely intertwining and anastomosing branches, is kept in mind.

Lastly, although, from a functional viewpoint, the "black line" bounded body of *P. squamosus*, immersed in the attacked wood, must be identified with the true sclerotia of, for example, *P. umbellatus*, it is necessary to distinguish these structures morphologically. On the distinction set out in a previous paper (8), that the term sclerotium should be reserved for such mycelial bodies as are borne free at the surface of the substratum, or are subsequently set free by the disintegration of the substratum in which the fungus originally developed, it is proposed to refer to the bodies formed by *P. squamosus* as pseudosclerotia.

VI. SUMMARY

The black lines of *Polyporus squamosus* have been found in the wood of elms. Isolations were made from these lines and an account of the development of *P. squamosus* in artificial culture is given. Particular mention is made of the formation of black plates, or lines as they appear in section, in culture media and of the appearance of abnormal fructifications. Pure cultures of the fungus on sterilized wood blocks have produced black lines in the wood, similar to those occurring in nature.

From a consideration of the structure and formation of the black plates, the suggestion is made that they form the limiting layer or rind of sclerotium-like bodies buried in the attacked wood.

A review is given of sclerotium formation and structure in several species of *Polyporus*, and analogies are made between these sclerotia and the sclerotium-like body of *P. squamosus*. It is decided to distinguish the *P. squamosus* body from these sclerotia by naming it a pseudosclerotium, on the criteria previously advanced for *Armillaria mellea* (8).

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EXPLANATION OF PLATES XVIII AND XIX

All figures refer to *Polyporus squamosus* (Huds.) Fr.

PLATE XVIII

Fig. 1. Inoculated pear wood block on malt-agar slant showing a series of dark lines in the luxuriant mycelium. Description in text. × ¾.

Fig. 2. Papillae produced at the margin of a malt-agar slant. × ¾.

Fig. 3. Inoculated sycamore block showing the formation of the black line to include the cotton-wool at the base. Note the copious exudation of dark-coloured drops. × ¾.

Fig. 4. A group of oidia stained with erythrosin. The thick wall and granular contents can be seen. × 350.

Fig. 5. The initial stages of black line formation in malt-agar culture. Note the palisade structure with the hyphae composing it all derived from one side. × 350.

Fig. 6. Section through the black line in wood from Yate. The typical massing of the bladder hyphae can be seen. × 350.

Fig. 7. Inoculated ash wood block after 5 weeks showing black lines and ovals. The superficial mycelium was removed before photographing. × ⅔.

PLATE XIX

Fig. 1. Culture on potato-dextrose agar. Note the dark plate covering the hyaline mycelium and the abnormal fructification at the upper end of the culture. Both are dusty in appearance due to the formation of oidia. × ⅔.

Fig. 2. Four months old cultures on malt-agar slants showing abnormal fructifications and the formation of papillae at the side of the slant and on one of the fructifications. × ⅔.

Fig. 3. Culture on "C" medium showing bracket-shaped body developed after 5 weeks' incubation. × ⅘.

Fig. 4. A later stage of the culture shown in fig. 3. Note the production of a number of curious, abnormal fructifications with finger-like projections. See text, p. 457. × ⅗.

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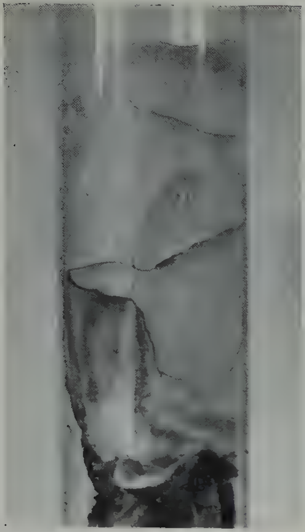

Fig. 1

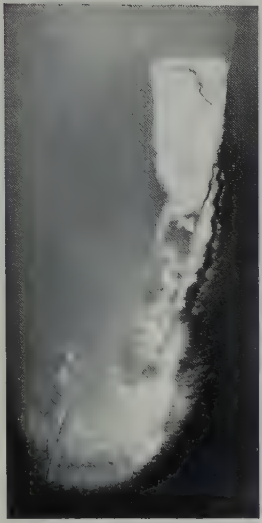

Fig. 2

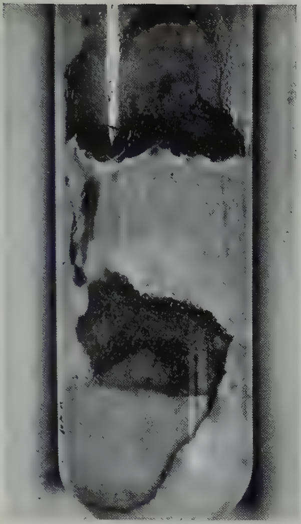

Fig. 3

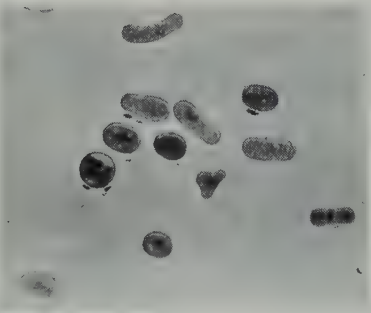

Fig. 4

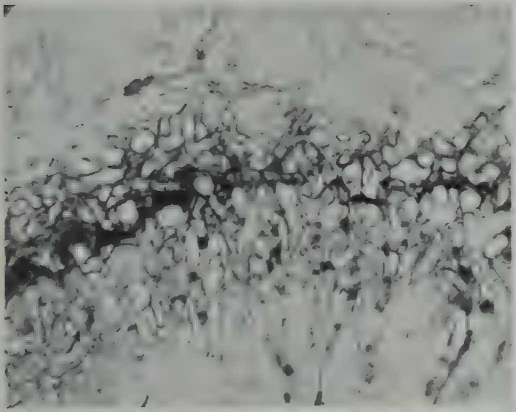

Fig. 5

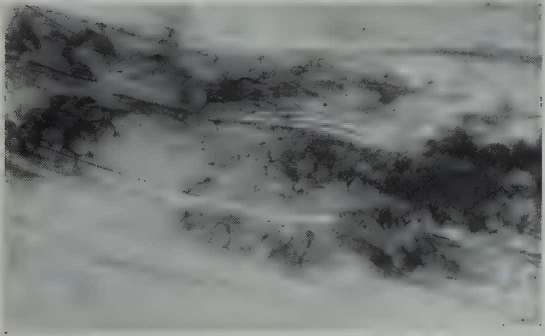

Fig. 6

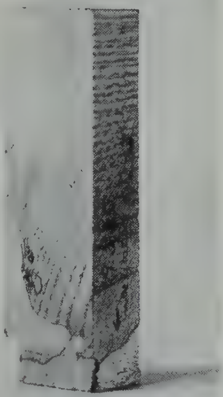

Fig. 7

CAMPBELL AND MUNSON—ZONE LINES IN PLANT TISSUES (pp. 453–464)

A STUDY OF *FOMES FRAXINEUS* AND ITS EFFECTS ON ASHWOOD

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(With Plate XX and 5 Text-figures)

INTRODUCTORY

FOMES FRAXINEUS (BULL.) FR. has a fairly wide distribution, being found in Europe and the United States of America. Lloyd (7) reported the fructifications as common in Europe on ash, apple and locust. According to Rea (13) the fructifications are uncommon in Britain, though they do occur on ash and laburnum. Massee (8) listed it as present only on old ash trunks. Recently Nicolas (11) described a case where he had found a fructification on elm at Toulouse, France. In the United States of America it has been reported by Kaufman (6) on ash in Michigan, in all cases on old stumps, also by Overholts (12) in Ohio, Illinois and Iowa on ash, and by Murrill (10) in New York and Louisiana on trunks and stumps of sweet gum (*Liquidambar styraciflua* L.) and peach, in addition to ash. Baxter (2) stated that it had been seen on oak and maple also, in Michigan. The fungus is not known to occur on coniferous wood. In the living tree the fungus causes a rot confined to the heartwood, but on the death of the tree, as when it is cut and worked, the fungus invades both sap and heartwood, causing a white rot. At the Forest Products Research Laboratory, Princes Risborough, the fungus has been isolated from the ashwood of a motor-'bus body.

The sporophore of the fungus was not found by the author, but it has been fully described by Rea (13) as follows: "Pileus 7–25 cm. *whitish becoming rubiginous and fuscous*, applanate, dimidiate, sometimes imbricate, glabrous, often coarsely tuberculated, margin incurved, pubescent at first. Tubes *pale cinnamon* 5–25 mm. long, stratose; orifice of pores *white, becoming greyish*, minute, round or oblong. Flesh *yellowish, somewhat zoned*, soft becoming hard and woody. Spores white subglobose $5–7 \times 6\,\mu$. Smell often strong, Jan.-Dec." *Fomes fraxinus* is synonymous with *Polyporus incanus* (Quél) and *Fomes cytisinus* (Berk.) Massee, Lloyd(7) considering the latter name to be the more correct one. The fungus is closely allied to *F. ulmarius* and has been confused with *F. fraxinophilus* in the U.S.A., but it is considered a distinct species by Von Shrenk(14).

Little work of an experimental nature has been done on the fungus, Baxter(2), alone, having described its appearance in culture. He gives a review of the literature relating to its occurrence in the field, and from his experiments he reports that little damage was done to ashwood by the fungus in three months.

The present investigation was suggested by the Mycology Department of the Forest Products Research Laboratory, Princes Risborough, to add to the existing knowledge of the organisms commonly attacking ashwood in this country. A culture was obtained from Princes Risborough, which had been originally cultured from a sporophore found on a living *Robinia pseudacacia* L. (locust tree). This culture was subcultured on to malt agar (3 per cent. Kepler's malt extract 2 per cent. agar) on which the fungus grew satisfactorily.

EXPERIMENTAL WORK

Description of the fungus in culture

The fungus was cultured on slopes of 2 per cent. and 5 per cent. malt agar in boiling tubes.

On 2 per cent. malt agar the growth proceeds from the inoculum as fine, radiating hyphae appressed to the surface of the medium. As time proceeds the mycelium thickens on the medium forming a snow white, subfelty to felty layer. At no time are aerial hyphae common. Branching of the hyphae is not frequent and only occurs at some distance from the growing-point and the new branch grows closely beside the parent hypha, not spreading sideways much but tending to a more or less parallel growth. When the culture was 1 week old, the centre had become coloured *Cartridge Buff*[1] (Ridg.) with parts showing *Pale Pinkish Buff*. At the end of a fortnight the colour had lightened, the growth finally forming a more or less white mat. This mat of mycelium had now covered the surface of the slant but did not extend much into the medium, being mainly limited to the surface. At the end of the third week, pore surfaces were forming at the upper end of the slope over an extensive area. These pores originated as small depressions scattered over portions of the mycelium, and later growth caused them to become somewhat deeper.

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[1] Colour names in italics are from Ridgeway's *Colour Standards and Colour Nomenclature*.

On 5 per cent. malt agar the growth proceeds in a very similar manner but at all times it is more compact. This is especially evident in the purer white appearance of the young culture. At the end of the first week the centre of the culture had become similarly coloured to that on 2 per cent. malt agar, but in this case the colour deepened so that at the end of a fortnight the colour was *Wood Brown* with a zone of *Pale Pinkish Buff* remaining at the lower end of the slope. This mat-like mycelium now covered the slope, being mainly present on the surface of the medium. At the end of 3 weeks pore surfaces were developed at the upper end of the slope in a number of tubes. These pore surfaces were more limited in area than those on 2 per cent. malt agar and were first formed as raised cushions of compact hyphae *Pale Pinkish Buff* in colour. The pores began as a series of depressions on the surface of these cushions, later extending some distance into them in a direction parallel to the force of gravity.

The fungus on both concentrations had a pronounced odour similar to that of *Agaricus campestris* (common mushroom).

Microscopic details of the hyphae

The young hyphae were dense and unvacuolated, averaging 2–5μ in diameter. Branching did not appear until about 700μ from the growing tip, and generally clamp connexions were also absent from this region. The branches emerged from just behind the septum.

The mature hyphae were of two types, (*a*) aerial hyphae, (*b*) submerged hyphae.

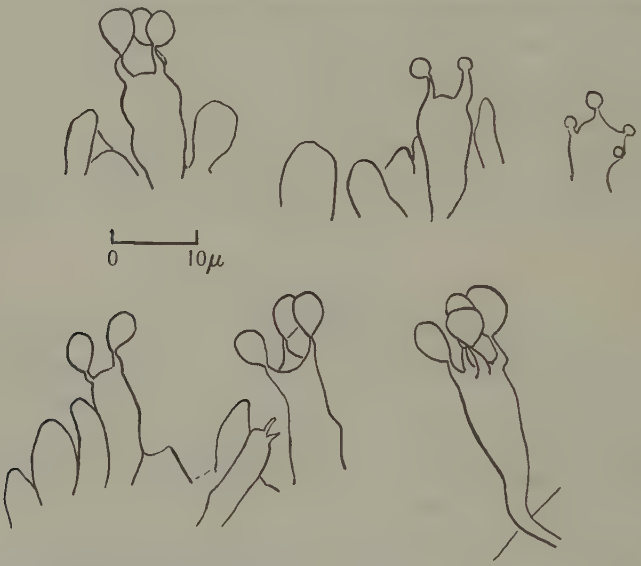
(*a*) The aerial hyphae were vacuolate, and hyaline with thick walls and little branching. The diameter of these hyphae varied very slightly, being generally 2μ. Cross walls were not seen except in hyphae close to the agar surface.

(*b*) The submerged hyphae were vacuolate, septate and had many clamp connexions. They varied considerably in diameter from less than 1μ up to 4μ, but the majority averaged 1·5–2·0μ. Branching was common. Many chlamydospores were present, generally being more numerous near the surface of the medium. They measured on average $13 \times 10\mu$, extremes measuring $19 \times 10{\cdot}5\mu$ and $10{\cdot}5 \times 9\mu$. The chlamydospore often appeared as a double spore without a dividing wall in the middle. The hyphae and chlamydospores contained many oil drops. The submerged type of hyphae was also found on the surface of the medium below the fibrous aerial hyphae.

The clamp connexions were of the usual type, and the "medallion" type found in many other wood-rotting basidiomycetes was absent. No crystals were found on the hyphae, but oblong and cubical crystals were common in the medium surrounding the hyphae. The crystals appeared as soon as the mycelium became dense.

Microscopic details of pore surfaces

Some of the pore surfaces, developed on the 5 per cent. malt agar, were fixed in acetic alcohol and imbedded in paraffin wax. Sections, 10μ (approx.) thick, stained in Delafield's haematoxylon, showed clearly that typical basidia, each bearing four basidiospores, were present. This is contrary to the observations made by Baxter (2) who reports that only secondary spores were produced in the pores. None of these secondary spores were seen by the author.



Text-fig. 1. Basidia with basidiospores produced in artificial culture.

The pore surfaces in culture varied in size from small cushions of mycelium with an average diameter of about 5 mm. up to large flat areas extending over almost the whole surface of the culture. The pores were oval with an average mean diameter of 0·24 mm. The longer axis of the orifice of the pore varied from 0·39 to 0·19 mm., and the shorter axis from 0·28 to 0·14 mm. In the case of one such pore surface there were twelve pores in a circle of 2 mm. diameter. The basidia ranged from

5 to 10 μ in diameter (at their widest point), with an average of 6 μ, and their length was more than 11 μ. This length was not accurately determined owing to the bases of the basidia being obscured in the very compact hymenium. In any single section only a few of the basidia were fully mature and bearing basidia at one time. The basidiospores were oval and measured $4 \cdot 4 \times 4 \cdot 0 \mu$ as an average, while extremes were found from $6 \cdot 0 \times 7 \cdot 5 \mu$ to $3 \cdot 0 \times 4 \cdot 0 \mu$. The hyphae present in the context ranged from 1·7 to 3·4 μ, with an average of 2·3 μ.

Monobasidiospore cultures

Single spore cultures were obtained from basidiospores by a modification of the method used by Bose (4) with fructifications. Petri-dish cultures bearing pore surfaces were left in an inverted position until the spores discharged on to the lid—generally 12–24 hours. Some spores were then removed from the lid with a sterile needle and streaked on a plate of 3 per cent. malt agar. This transference of the spores was facilitated by the presence of a small amount of water of condensation on the lid. The process was repeated a number of times and the plates incubated. Most of the spores germinated within 24 hours, and monosporous mycelia in isolated positions were transferred to tubes of malt agar. Six days elapsed from the time of inoculation until these primary hyphae became visible to the naked eye. In all, ten monosporous cultures were obtained and labelled A–K.

The growth characters of one of these monosporous cultures (E) and of the original diploid culture were compared on 2 and 5 per cent. malt-agar slants in boiling tubes. At both concentrations, the basidiospore culture grew more profusely, forming a woolly mycelium which covered the slant in 3 weeks. The colour of the basidiospore culture was slight and finally ranged from *Capucine Buff* to *Pale Ochraceous Buff*.

Variation in the appearance of these monospore cultures suggested a grouping on this basis. Thus cultures B, F, H and K showed a sparse fluffy growth, while A, C, D, E, G and I showed thick fluffy growth. It was found that no correlation existed between this grouping and the grouping according to sexual reaction.

To divide the cultures into their respective sexes a series of 100 Petri dishes of 3 per cent. malt agar was inoculated with all the possible pairings of these monospore cultures in duplicate. When the mycelia from the two inocula in each dish had intergrown, after approximately 3 weeks, thin vertical sections were cut from the region of intermingling and stained with cotton blue in lactophenol, which picked out the hyphae very plainly, rendering clamp connexions clearly visible when present. The presence of clamp connexions was taken to indicate a successful pairing resulting in a diploid mycelium. The result of this experiment is shown in Table I.

Table I

Experimental pairing of monospore cultures

	A	F	G	C	H	K	B	E	D	I
A	0	0	0	+	+	+	0	0	0	0
F	0	0	0	+	+	+	0	0	0	0
G	0	0	0	+	+	+	0	0	0	0
C	+	+	+	0	0	0	0	0	0	0
H	+	+	+	0	0	0	0	0	0	0
K	+	+	+	0	0	0	0	0	0	0
B	0	0	0	0	0	0	0	0	+	+
E	0	0	0	0	0	0	0	0	+	+
D	0	0	0	0	0	0	+	+	0	0
I	0	0	0	0	0	0	+	+	0	0

Each + represents a successful pairing resulting in a diploid mycelium. Thus there are at least four different types of basidiospore. This indicates a two-factor basis of sex determination, possibly of the *Aa Bb* type as is found in *Coprinus lagopus* and in many other basidiomycetes. This seems to be further supported by the fact that 26 per cent. of the pairings produced diploid cultures. When two factors are concerned 25 per cent. of the pairings are successful.

Factors influencing the production of fructifications

Medium.

The following range of media was prepared: 2 per cent. malt-extract agar, potato-extract agar (200 g. potato per litre), prune-extract agar (20 g. prune per litre), oatmeal agar (60 g. ground Quaker Oats per litre) and ashwood-extract agar (50 g. fine shavings of ashwood per litre). All these were sterilized by autoclaving for 20 min. at 15 lb. pressure, and six plates of each were poured. When cold these were inoculated in the centre with the fungus, and at the end of 1 month at room temperature, in the light, the following observations were made:

2 per cent. malt-extract agar. A ring of pore surfaces formed in each plate at a distance of approximately 2 cm. from the inoculum. Basidiospores produced.

Ashwood-extract agar. Vigorous mycelial growth but no pore surfaces.

Potato-extract agar. Sparse mycelial growth forming very diffuse pore surfaces in all plates, few pores being fully formed. The appearance was more of knots of hyphae. No basidiospores were observed.

Oatmeal agar. Very vigorous mycelial growth forming a thick felt with pores appearing as minute corroded pits scattered on the surface. No basidiospores were observed from these pores.

Prune-extract agar. Vigorous growth eventually forming an unevenly thickened felt. A very well-developed pore surface producing basidiospores was formed on one of the plates, but no pore surfaces were developed on the other plates.

A prune-extract agar was prepared with the addition of an aqueous extract of barley, as it was thought that the soluble proteins of barley might aid sporophore production. No pore surfaces were developed on this agar.

A prune-extract agar prepared according to the method of Mounce (9), 1 litre containing extract from 120 g. of dried prunes, supported vigorous mycelial growth, and extensive pore surfaces and basidiospores were produced at laboratory temperature (± 18° C.) in the light.

Moisture following desiccation.

Some old cultures, $5\frac{1}{2}$ months old, which had been allowed to become hard and dry were used as inoculum for inoculating fresh 2 per cent. malt-agar slopes. Pore surfaces were produced in some cases, but not more frequently than was usual with normal inoculum.

Light and temperature.

A series of cultures on 2 per cent. malt agar in boiling tubes was subjected to a variety of treatments as shown in Table II. Most of the cultures eventually produced pore surfaces irrespective of the treatment they had received.

Table II

Effect of temperature and light

	Number of replicates	Temperature	Illumination	Observations on 23 Feb.	24 May	5 Nov.
A	6	Room temp. (about 18° C.)	Light	Culture not coloured	No fruct.	Fruct. in 4
B	6	Room temp. (about 18° C.)	Dark	Culture not coloured	No fruct.	Fruct. in 5
C	6	25° C. for 14 days, then about 18° C.	Light	Culture coloured	No fruct.	Fruct. in 6
D	6	25° C. for 14 days, then about 18° C.	Dark	Culture coloured	No fruct.	Fruct. in 4

The presence of colour in C and D seems to be due to a staling action. These cultures had grown more rapidly than the others of the series because they were incubated at 25° C.

During the period from September 1933 to May 1934 no fructifications were produced on any of the cultures (with the exception of one culture on prune-extract agar prepared according to Mounce(9)). No adequate explanation for this can be put forward, as they were readily obtained during the period February 1933 to September 1933. The culture does not seem to have "gone off" through age, as fructifications were again produced during the summer of 1934.

Effect of temperature upon the rate of growth

A series of Petri dishes containing 25 c.c. of 2 per cent. malt agar (*p*H 6·8) was inoculated with transplants, measuring approximately 4 mm. square, from a young Petri-dish culture of the fungus.

Three dishes were placed in incubators at each of the desired temperatures, and measurements of the increments in average diameter of each culture were made daily. Since these increments were very variable for the first 3 days the results presented in Table III omit these increments from the average.

Table III

Effect of temperature on rate of growth. Average daily increment in diameter for each temperature

Temperature in ° C.	10	15	20	25	26	28	31	33
Rate of growth in mm. per day	1·0	2·4	4·6	8·2	8·6	8·3	3·7	0·0

Thus the optimum lies about 26° C. and the maximum at 33° C. No attempt was made to determine exactly the lower limit for growth, but it was found that growth was very slow below 10° C. The figures show that the fungus would be classed as moderately quick growing on agar as compared with other wood-decaying fungi.

In a comparative test it was found that both diploid and haploid cultures showed the same growth-rate. This is contrary to what Buller(5) states, i.e. that the diploid culture grows twice as fast as the haploid. Both cultures grew more rapidly than *Fomes fraxinophilus*.

Effect of hydrogen-ion concentration upon the rate of growth

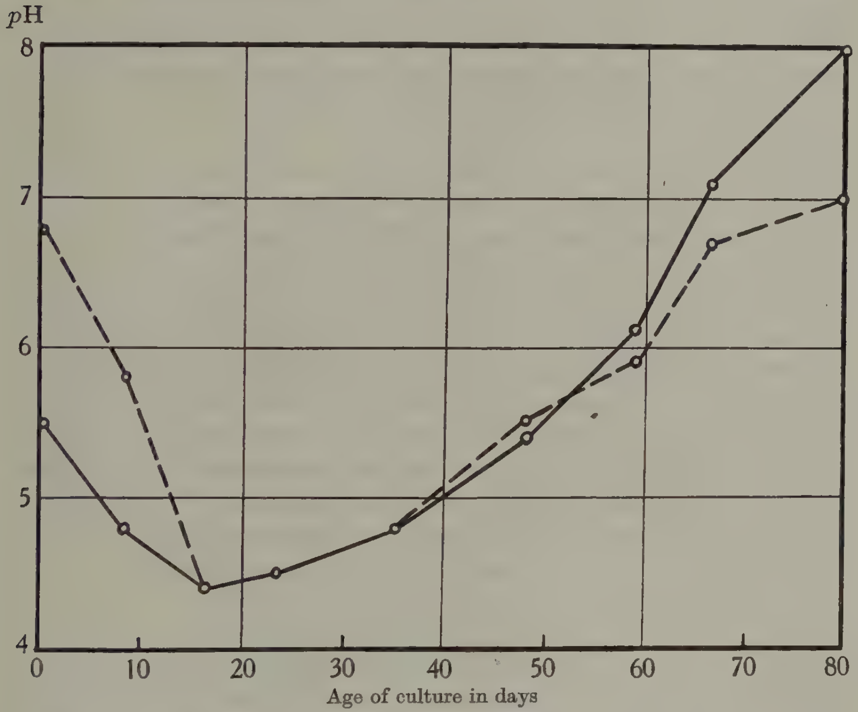
A 2 per cent. malt agar was prepared and either malic acid or sodium carbonate added to 100 c.c. portions so that a range of *p*H from 2·0 to 9·6 was obtained. Four Petri dishes, each containing 25 c.c. of medium, were poured with each portion. When these media had solidified each dish was inoculated in the centre with a 4 mm. square of culture of the fungus and incubated at 20° C. The daily increments were averaged and are presented in Table IV.

Table IV

Effect of hydrogen-ion concentration upon the rate of growth. Average daily increment in diameter for each pH

pH	2·4	3·5	4·0	5·0	6·0	6·8	7·0	8·0	9·2	9·4
Rate of growth in mm. per day	0·0	1·4	3·5	4·4	5·5	5·3	5·0	4·3	0·5	0·0

From this range it will be seen that pH 6·0 gave the optimum growth. The daily increments were observed to increase as the culture became older in all cases where the pH was 6 or over, and to decrease with age where the pH was below 6. The final pH of each of the cultures was determined by a colorimetric method, and it was found that the hydrogen-ion concentration had risen in all. For this reason the growth figures presented do not express exactly the growth rate at the pH stated and are merely comparative.



Text-fig. 2. Graph showing changes in pH of two series of 2 per cent. malt extract cultures during the growth of the fungus.

To investigate the changes in pH undergone during the growth of the fungus, six flasks, each containing 60 c.c. of 2 per cent. malt-extract liquid were sterilized by autoclaving, and the *p*H of the extract was found to be 6·8. The *p*H of three of these flasks was then adjusted to 5·5 by the addition of small quantities of 10 per cent. malic acid solution (sterile). Each was then inoculated with 4 mm. squares of *F. fraxineus* culture, care being taken not to allow any of the inocula to become submerged. Each week small amounts of the medium were withdrawn and the *p*H determined. These readings were averaged for each set of three and plotted on a graph (Text-fig. 2). It will be seen that the hydrogen-ion concentration rises for the first fortnight and then commences to fall until the cultures become near or even slightly alkaline. It is suggested that after 2 weeks some autolytic process goes on with the formation of NH_3 which causes a reduction in the concentration of hydrogen ions.

Enzyme study

The fungus was grown for 1 month in conical flasks containing 2 per cent. malt-liquid medium. The mycelium was collected and washed thoroughly in running water for several hours. This washing was followed by a short washing in distilled water and the mycelium was then dried in a desiccator and finely ground with an equal weight of silver sand in a mortar. An enzyme extract was prepared from this powder as required, by suspending 1 g. of the powder in 100 c.c. of distilled water and leaving it for 2 hours with frequent shaking. The suspension was then centrifuged and the clear liquid decanted. For all the following tests with this extract a control extract was prepared by autoclaving a portion of the enzyme extract. The results have been summarized in Table V.

Table V

Production of enzymes by the fungus

Enzyme	Substrate	Test	Reaction
Hydrolysing enzyme (diastase)	1 % starch solution	Reduction of Fehling's solution	+
Hydrolysing enzyme	Hemicellulose (paragalactan)	,, ,, ,,	–
Hydrolysing enzyme	Alpha cellulose	,, ,, ,,	–
Glucoside splitting enzyme (emulsin)	Amygdalin	,, ,, ,,	+
Glucoside splitting enzyme (emulsin)	Salicin	,, ,, ,,	?
Invertase	1 % sucrose	,, ,, ,,	+
Zymase	1 % glucose	Production of CO_2	+
Pectinase	Potato discs	Loss of cohesion	+
Catalase	Hydrogen peroxide	O_2 formation	+
Oxidase	Guaiacum + H_2O_2	Blue colour	+
Peroxidase	Guaiacum	Blue colour	+
Lipase	Olive oil	Formation of glycerol	+

Where a plus sign occurs in the "Reaction" column it indicates that the enzyme extract gave a more vigorous action than the control, indicating the presence of the particular enzyme tested for. A minus sign indicates no action more vigorous than the control and suggests the absence of the enzyme sought.

It will be seen that the predominating enzymes are of oxidizing and hydrolysing nature, and that the oxidizing are the more abundant.

Effect of nitrogen on the activity of the enzymes

As it had been noted that the fungus could grow on a variety of artificial media and also on many timbers under laboratory conditions, although it is confined to only a few timbers under natural conditions, experiments were made to ascertain whether nitrogen might be the limiting factor for the growth of the fungus. Since the decay of wood is thought to be effected mainly by hydrolysing and oxidizing enzymes, a representative of each of these two groups was tested, diastase and oxidase being selected as the test enzymes. In all cases the enzyme extract was prepared from powdered mycelium.

The fungus was grown on liquid synthetic media made to standard formulae, in which the quantity of nitrogen was varied, but growth was poor on all. The media included Brown's, Dox's and Czapek's Solution II. The latter, modified by the use of neutral potassium phosphate (K_2HPO_4) instead of the acid potassium phosphate (KH_2PO_4), gave a pH of 7·0 and supported the best growth. The sources of nitrogen included asparagin, sodium nitrate and ammonium nitrate used at various concentrations. The activity of the diastase was determined by the hydrolysis of starch, while the activity of the oxidase was determined by a comparison of the rates at which gum guaiacum was turned blue. Both methods gave comparative results only.

The indications from these experiments were that the complete absence of nitrogen caused a great decrease in the activity or the amount of the enzymes studied, but that increasing the amount of nitrogen at the concentrations tested had no effect. The lowest concentrations of nitrogen sources tested were 0·05 per cent. asparagin, 0·05 per cent. sodium nitrate and 0·025 per cent. ammonium nitrate; thus the concentrations where nitrogen is a limiting factor probably lie below these concentrations. The effect of nitrogen on the activity of the enzymes seemed to be correlated with its effect on the growth of the fungus.

Resistance to fungicides

The resistance of the fungus to fungicides was determined by means of agar tests, using *Fomes fraxineus* as the test fungus. The preservatives used were zinc chloride, sodium fluoride and coal-tar creosote (British standard specification type A), and in each case various quantities of the preservatives were added to 20 c.c. portions of 2 per cent. malt agar after autoclaving and the total volume of mixture made up to 25 c.c. by the addition of water. This mixture was then poured into Petri dishes and inoculated, when cold, with small transplants (5 mm. square approx.). Each experiment was done in four replicates. The concentrations varied from 0·005 to 2·2 per cent. for zinc chloride, 0·005 to 0·5 per cent. for sodium fluoride and 0·04 to 0·4 per cent. (by weight) for creosote. The toxic point was taken as the concentration at which no growth occurred, and these results are summarized in Table VI.

Table VI

Resistance of the fungus to preservatives in agar culture

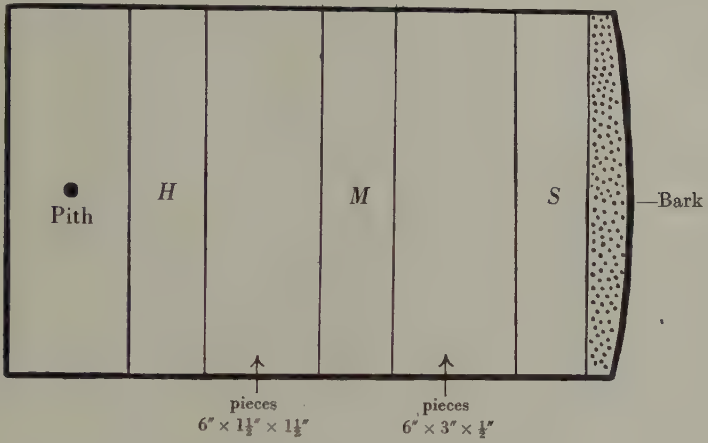
	Zinc chloride %	Sodium fluoride %	Coal-tar creosote %
Toxic point	0·1	0·09	0·16
Next concentration at which growth occurs	0·07	0·08	0·12

From the figures in Table VI it will be seen that *F. fraxineus* is only moderately resistant to antiseptics and should be fairly readily controlled by the use of them.

Growth of the fungus upon wood

Ashwood placed on a culture of the fungus on agar rapidly became covered with a white felt of mycelium which could be easily stripped off in a sheet resembling soft kid leather, leaving the wood below quite clean. Even in late stages of decay there is little appearance of the decay on the surface except for some shrinking and longitudinal splitting on drying, but the interior of the block becomes much rotted, showing a characteristic "brashness".

Small blocks of ashwood (*Fraxinus excelsior* L.) were used as substratum for the fungus to determine the rate of decay under certain conditions. The ashwood, obtained through the Forest Products Research Laboratory, Princes Risborough, came from a tree grown on poorly drained soil at East Moor, Mapledurwell, Hants, and felled on 20 December 1932. The samples used were all sound and were obtained from the main trunk about 4 ft. above ground-level. In cutting up the wood, sapwood (S), heartwood (H) and intermediate wood (M) were kept separate. Small samples measuring $3 \times 1 \times \frac{1}{2}$ in. were cut from these regions. From wood between these areas larger pieces were cut $6 \times 1\frac{1}{2} \times 1\frac{1}{2}$ in. and $6 \times 3 \times \frac{1}{2}$ in. This method of cutting is indicated in Text-fig. 3.

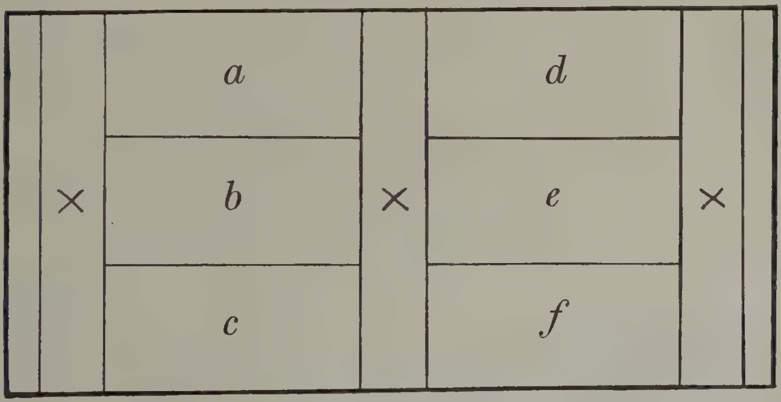


Text-fig. 3. Transverse section of trunk.

Growth upon seasoned and unseasoned ashwood

As the wood was quite fresh when received, some 2 months after felling, the decay of the fresh wood was compared with the decay of some of the same wood seasoned for 2 months in the laboratory.

Two pieces, $6 \times 3 \times \frac{1}{2}$ in., were sawn up as shown in Text-fig. 4.



Text-fig. 4.

Both sets of test samples (*a*, *b*, *c*, *d*, *e*, *f*) were weighed and autoclaved for 20 min. at 15 lb. pressure, then placed on cultures of the fungus in boiling tubes and incubated at 26° C. The moisture content of the thin strips of wood (*X*, *X*, *X*) was determined and the oven-dry weight of the test samples calculated by assuming that they had the same moisture content as the small strips (*X*, *X*, *X*) from the same piece. It was realized that this method only gives an approximation. At the end of 3 months at 26° C. the blocks were taken from the cultures and the surface mycelium removed. They were then weighed, dried in the oven and weighed again. In Table VII the percentage loss of dry weight is shown and also the final moisture content expressed as a percentage of the dry weight. The average loss of dry weight is seen to be 10 per cent.

Two other pieces, 6 × 3 × ½ in., were also sawn up in the same way at the same time as the previously mentioned pieces, but the test samples, in this case, were kept exposed in the laboratory for 2 months to season them. They were then dried in the oven and weighed. The moisture content of each block was adjusted to approximately the same as that of the fresh specimens by the addition of a suitable amount of distilled water, which was readily absorbed. They were then treated as before and at the end of 3 months weighed as before. These figures are shown in Table VIII. The average loss of weight in this case was 6·5 per cent. This shows a considerable decrease in the amount of decay caused in seasoned as compared with unseasoned wood under the conditions of the experiment.

Table VII

Percentage loss of weight during 3 months of fresh ashwood blocks 2 × 1 × ½ in.

Sample		*a*	*b*	*c*	*d*	*e*	*f*	Aver-age	Combined average % loss of wt.
Piece 1	% loss in dry wt.	7·5	16·5	11·0	7·4	9·2	8·7	10·1	**10·0**
	Final % moisture	36·2	121·1	66·6	80·4	65·8	67·6		
Piece 2	% loss in dry wt.	12·6	7·7	9·3	10·7	9·4		9·9	
	Final % moisture	55·3	63·5	123·1	44·2	61·4			

Table VIII

Percentage loss of weight during 3 months of ashwood blocks 2 × 1 × ½ in. seasoned for 2 months

Sample		*a*	*b*	*c*	*d*	*e*	*f*	Aver-age	Combined average % loss of wt.
Piece 1	% loss in dry wt.	4·4	5·6	7·6	4·8	6·4	7·9	6·1	**6·5**
	Final % moisture	73·5	76·3	87·9	83·1	70·1	95·2		
Piece 2	% loss in dry wt.	7·4	8·1	4·9	8·9	6·9	5·3	6·9	
	Final % moisture	77·3	86·6	75·7	97·0	73·8	67·3		

Growth upon sapwood, heartwood and intermediate wood

Sample blocks of ashwood measuring $3 \times 1 \times \frac{1}{2}$ in. obtained from regions *S*, *M* and *H* (see Text-fig. 3) were used in this experiment. Before use some of the wood from each region was inspected for starch by staining the surface of the wood with several drops of iodine solution. It was found that the sapwood (*S*) showed a deep blue colour, the heartwood (*H*) showed no blue colour, while the intermediate wood (*M*) showed a very slight bluishness. Sections 15–20μ were cut on a Jung sliding microtome and starch grains were observed plentifully in the medullary ray cells and companion cells of the sapwood. Some of the cells were completely filled with starch grains. In the heartwood sections no starch grains were seen, and in the intermediate wood a few starch grains were visible in the medullary ray cells but not elsewhere. Tyloses were seen in some of the vessels of the heartwood but were not common.

The sample blocks were then numbered, oven-dried and weighed and, after autoclaving, were placed on active cultures covering the surface of 2 per cent. malt agar in potato dishes. To obviate the large variations in age from place to place on these cultures that would have resulted from the use of a single inoculum on the agar at the start, a number of inoculations had been planted over the surface of the agar at intervals of approximately 2 in. This gave a complete cover of mycelium within 1 month. Decay of the wood was permitted to go on for periods of 2, 4 and 6 months, at 26° C., and uniform conditions for each period of testing were maintained by placing all the blocks for each period in a single potato dish. Thus each of the three potato dishes contained six blocks of sapwood (*S*), six of heartwood (*H*) and six of intermediate wood (*M*). The blocks were arranged standing on one narrow side, and care was taken to see that every block was in contact with the surface of the culture.

At the end of 2 months all the blocks were removed from one of the potato dishes, oven-dried and weighed and the loss in dry weight calculated. By an oversight a weighing of the blocks before drying was omitted, and so the final moisture content of these blocks could not be found. In the subsequent series, removed at 4 and 6 months, this weighing was carried out. The results of this experiment are given in Table IX.

It will be seen from Table IX that although the sapwood lost weight during the first 2 months to the extent of 6·9 per cent., the intermediate wood lost only 1·5 per cent. and the heartwood only 0·6 per cent. in the

Table IX

Decay of ashwood samples $3\times 1\times \frac{1}{2}$ *in. during* 2, 4 *and* 6 *months*

	Sapwood		Intermediate wood		Heartwood	
Duration of decay	% loss of wt.	Final % moisture	% loss of wt.	Final % moisture	% loss of wt.	Final % moisture
2 months	5·9	—	1·6	—	0·6	—
	7·2	—	0·6	—	0·8	—
	5·6	—	1·3	—	0·1	—
	5·9	—	1·0	—	0·7	—
	11·1	—	2·1	—	0·7	—
	5·7	—	2·1	—	0·9	—
Average	**6·9**		**1·5**		**0·6**	
4 months	15·2	100·2	13·0	100·6	7·6	71·8
	14·4	113·7	11·0	78·2	7·3	76·0
	16·1	126·8	10·3	88·6	10·4	110·6
	15·9	118·8	10·3	78·5	7·6	73·2
	15·9	119·6	10·6	88·9	7·6	74·6
	15·7	106·7	8·9	66·3	8·9	97·2
Average	**15·5**		**10·7**		**8·2**	
6 months	17·7	55·7	11·8	49·2	10·3	45·7
	16·4	52·4	13·0	51·5	8·5	46·9
	19·7	68·8	16·9	60·2	14·5	57·8
	22·4	71·5	14·0	51·8	13·9	54·5
	19·9	65·7	11·8	50·2	19·1	86·6
	17·3	54·2	16·9	55·9	15·7	52·2
Average	**18·9**		**14·1**		**13·7**	

same time. This large difference in the loss in weight was not maintained during subsequent months. Thus, if the percentage weight lost in the first 2 months is subtracted from the percentage weight lost in 4 months, it will be seen that the decay in the second 2 months was fairly uniform, being 8·6 per cent. for sapwood, 9·2 per cent. for intermediate wood and 7·6 per cent. for heartwood. By a similar calculation the figures for the final 2 months can be found and they are 3·4 per cent. for sapwood, 3·4 per cent. for intermediate wood and 5·5 per cent. for heartwood.

The initial high loss in weight in sapwood caused by the fungus seems to be correlated with the presence of starch in the wood. When the supply of starch is exhausted the loss in weight is not significantly different for sapwood, heartwood or intermediate wood.

Growth upon other woods

Small blocks of various woods not generally attacked by the fungus were oven-dried and weighed, then autoclaved and placed on cultures of the fungus in special culture flasks and left to incubate at 26° C. for 3 months. The blocks were then removed, weighed, dried and reweighed. It was found that the fungus could cause considerable decay in all the woods tested and the summarized results are presented in Table X, each figure being the average of six blocks.

Table X

Average loss of weight of autoclaved wood samples after 3 *months*

	Average dry wt.	Average % moisture (final)	Average % loss of wt.
Oak (sap)	6·24	66·9	4·9
Oak (heart)	9·71	40·3	1·1
Sitka spruce	4·53	55·5	1·8
Beech	14·55	45·2	9·3
Scot's pine	5·44	32·8	4·5
Elm (sap)	6·97	36·9	9·3
Elm (heart)	7·35	67·2	3·3

Since it was considered possible that the autoclaving was altering these woods fundamentally, blocks of each of these woods were placed standing on a pad of absorbent cotton-wool saturated with water in a bottle and were sterilized by steaming for 1 hour on three successive days. The blocks were then inoculated with a single small piece of mycelium from an agar culture inserted between them and incubated at 26° C. At the end of 8 months it was found that all the woods had lost weight, as shown in Table XI. The final moisture contents of the woods were irregular, and this seems to have been influenced by the relative amounts of decay. The figures are only of interest inasmuch as they show that some loss of weight occurred in each case.

Table XI

Loss of weight of steam-sterilized wood samples after 8 *months*

	Dry wt.	% moisture (final)	% loss of wt.
Oak (heart)	9·66	20·0	5·3
Sitka spruce	4·59	38·8	4·0
Beech	9·93	126·0	39·2
Scot's pine	5·47	74·2	16·1
Elm (sap)	6·41	141·9	33·4
Elm (heart)	7·46	92·5	18·4

Growth upon Robinia pseudacacia

Two samples of *Robinia pseudacacia*, one an old specimen from Kew and the other an air-dried specimen from the Forest Products Research Laboratory, were sawn into blocks 10 × 2·5 × 1·5 cm. After drying they were weighed, autoclaved and placed on cultures of the fungus. After 3 months' incubation at 26° C. the final moisture content and the loss of weight were found. Those figures are summarized in Table XII, each figure being the average of five blocks. It is interesting to note that although this culture of the fungus was originally isolated from *Robinia pseudacacia*, yet it caused less loss of weight in this wood than in ashwood.

Table XII

Average percentage loss of weight of Robinia *samples after* 3 *months*

	Average dry wt.	Average final moisture, %	Average % loss of wt.
Kew specimen	22·31	30·3	0·66
F.P.R.L. specimen	18·39	35·9	0·70

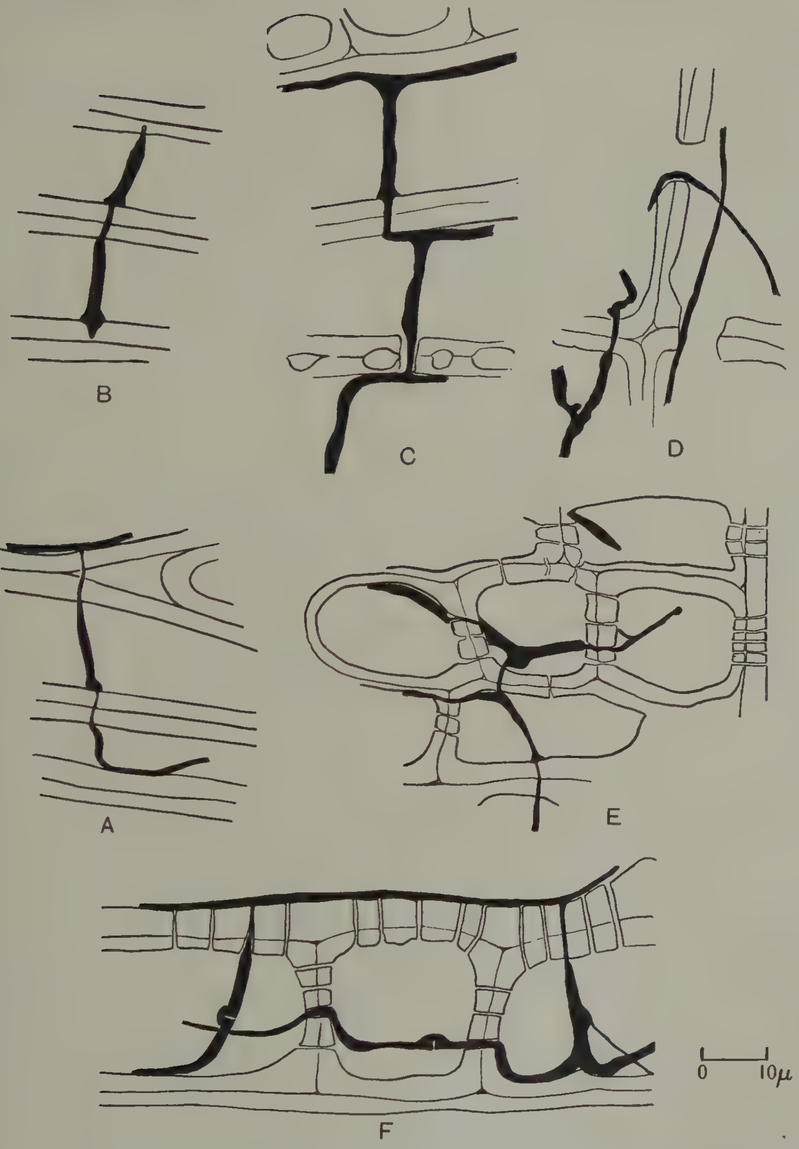
Appearance of the mycelium in wood and its effect on the wood cells

Small blocks of ashwood, trimmed up to true radial, tangential and transverse faces, were autoclaved and placed on active cultures of the fungus. Sample blocks were removed at weekly intervals during a period of 2 weeks and sections were cut 15–20μ thick with a Jung microtome. To facilitate the sectioning the blocks were soaked in a mixture of equal parts of glycerine, spirit and water for several days according to the method of Baxter (3). Penetration was assisted by evacuating the vessel with a vacuum pump for several minutes after the blocks were placed in the mixture. The sections were stained in safranin and picro-aniline blue. It was found that the fungus travelled freely along the vessels, forming a tangled mass of hyphae. From the vessels it spread through the wood in all directions, frequently penetrating the walls of the tracheids. It was in the medullary ray and wood parenchyma cells only that the hyphae were seen to utilize the pits; elsewhere any point in the cell wall appeared to be suitable for penetration. The hyphae ranged from 1 to 7μ, but the most common diameter was 2μ. Chlamydospores were not commonly produced in the wood except in the vessels and especially at the exposed ends of the vessels, but at no time were they plentiful in these blocks. Typical clamp connexions were freely formed. In penetrating a cell wall the hypha first appears constricted to a very narrow thread in the wall, with an appressorium-like swelling against the surface of the wall. Later this hypha swells up to normal size and finally is seen lying loose in a bore hole somewhat larger than the hypha itself.

The appearance of the decay caused by the fungus in various woods

These blocks of wood varied in size from 2 to 3 c.c. and were placed on active cultures of the fungus for 4 months. The appearance of the blocks at the end of this period is as follows.

Ash. Little or no sign of decay on the exterior, but the interior extensively rotted and crumbly, showing a typical "brash" fracture.



Text-fig. 5. A to D. The fungus penetrating wood cells, with large bore-holes visible in D. E. Utilizing pits in walls of wood parenchyma cells. F. Utilizing pits between a vessel and wood parenchyma cells. A to E, tangential sections; F, radial section.

Beech. Soft and showing cracks running up the spring wood dividing off plates of summer wood. Great tendency for the wood to divide into fibres.

Oak sapwood. Soft and spongy. Large vessels filled with hyphae so that they appear white, macroscopically. Colour of wood lighter with a tendency for the wood to divide into fine fibres.

Oak heartwood. Loss of colour and softening of the exterior. The block was not spongy, however, and preserved its shape.

Scot's pine, sapwood. Soft and spongy. Extensive rotting on exterior. Tendency to break into fine fibres.

Sitka spruce. Firm with little sign of decay except for slight softening of exterior.

Effect of moisture in the wood on the rate of growth of the fungus

Rods of ashwood $6 \times \frac{1}{2} \times \frac{1}{2}$ in., cut with the grain, were sterilized by autoclaving and then coated on their sides with paraffin wax. The ends were not coated. These rods were stood upright on an active culture of the fungus in a suitable vessel and incubated at 26° C. It was assumed that a gradient of moisture would be formed between the lower end in contact with the agar and the upper end exposed to the air. One piece of wood was removed each week and sawn into $\frac{1}{2}$ in. cubes, numbered in series. Each cube was then split in half (approx.) longitudinally and one half used to determine the moisture content. The other half of each block was assumed to have approximately the same moisture content, and it was soaked in the softening mixture and sectioned. The sections were examined for the spread and stage of growth of the fungus.

During the first month the fungus scarcely progressed beyond the first cube. This slow growth was possibly caused by the relatively slow rate at which moisture passed up the wood. Thus, after 1 month, the moisture content of the wood in the third cube from the agar was only 23 per cent., whereas the cube in contact with the agar had a moisture content of 113 per cent. Sections from later rods revealed the fact that vigorous growth prevailed between the moisture contents of 87·5 and 36·4 per cent. In these rods many chlamydospores were present throughout the first cube, where the moisture content was 96–103 per cent., and few hyphae remained vigorous in this cube. Hyphae were also scarce where the moisture content was 36·4 per cent., and again chlamydospores were visible. Chlamydospores seem to be produced when conditions are either too dry or too moist for the hyphae to flourish.

This method has not given the desired information, as the fungus tended just to keep pace with the spread of moisture suitable for its growth.

Lethal temperature

Preliminary experiments with 1-month-old cultures of the fungus on 2 per cent. malt agar in tubes showed that there was considerable variation of the resistance of the fungus to heat. It was found that inocula from the upper end of a sloped tube, where drier conditions existed, were viable after longer periods of exposure to heat than inocula from the middle of the slope where more moisture was present. It is therefore important in all work of this kind that the inoculum should be taken from a standard position in the slant.

From such tube-culture experiments the fungus was found to withstand 40° C. for several days, but it was killed in 4 hours at 60° C., when the inoculum was taken from the middle of the slant. The time required to kill the fungus in timber may be longer and probably depends greatly on the moisture content. Further work has been done on these lines, and the results will be published separately.

SUMMARY

1. An account is given of the occurrence and distribution of *Fomes fraxineus*.

2. The appearance of the fungus in culture and details of the hyphae are described.

3. Fruit bodies, in the form of pore surfaces bearing basidiospores on typical basidia, were obtained. Monosporous cultures derived from these spores were found to be heterothallic, sex appearing to be determined by two factors.

4. The formation of fructifications is favoured by certain media but does not seem to be influenced by light, temperature or moisture.

5. The optimum temperature for growth is 26° C., and the optimum hydrogen-ion concentration is *p*H 6.

6. The fungus was found to be moderately resistant to zinc chloride, sodium fluoride and coal-tar creosote.

7. A list of enzymes found to be present is given and some preliminary experiments on the effect of nitrogen on the activity of diastase and oxidase produced by the fungus are outlined.

8. The growth of the fungus on ashwood and some other woods was investigated. The fungus grows well on ash, beech and elm and is able to attack oak, Scot's pine, Sitka spruce and *Robinia pseudacacia*.

A description of the hyphae in the cells of the wood and their effect upon the walls is given.

ACKNOWLEDGEMENTS

The writer is indebted to Prof. W. Brown under whose direction this work was carried out, and he wishes to express his gratitude to Mr W. P. K. Findlay for suggesting the problem and for helpful criticism and advice during the course of the work.

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EXPLANATION OF PLATE XX

Fig. 1. Pore surfaces forming on a 5 per cent. malt-agar slope (natural size).

Fig. 2. Basidium bearing basidiospores, from a pore surface produced on 5 per cent. malt agar (×900).

Fig. 3. Naturally occurring fructification of *Fomes fraxineus* formed on *Robinia pseudacacia*.

(*Received* 13 *November* 1935)

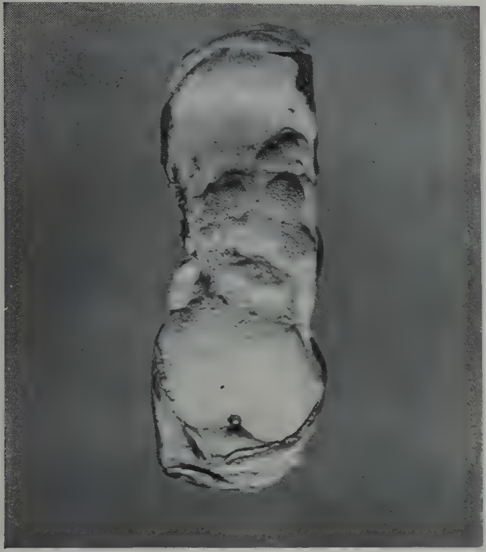

Fig. 1

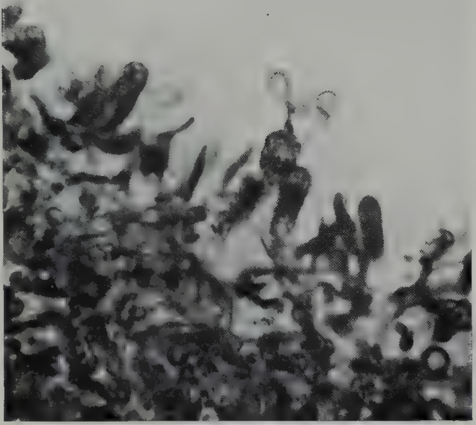

Fig. 2

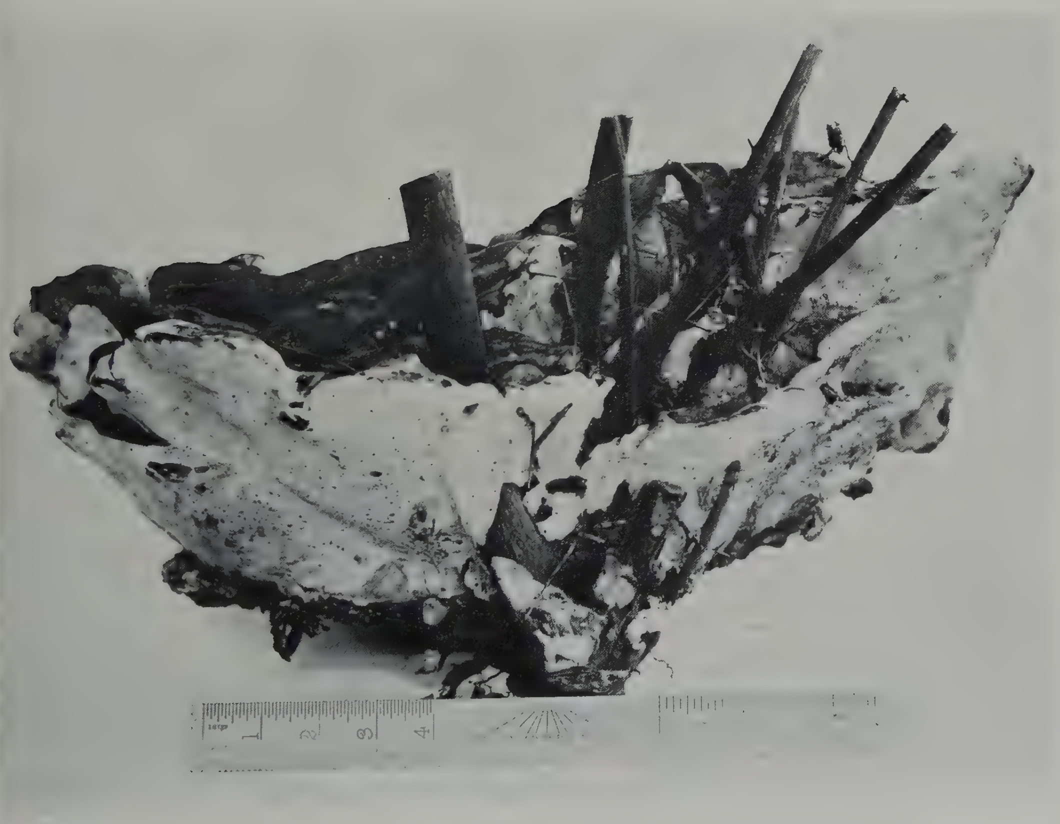

Fig. 3

MONTGOMERY—A Study of *Fomes fraxineus* and its Effects on Ashwood (pp. 465–486)

THE VIRUSES CAUSING TOP NECROSIS (ACRONECROSIS) OF THE POTATO

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THE naming of viruses on the basis of the symptoms that they cause in infected plants has been responsible for much of the confusion and apparent contradictions in potato-virus work. With the growth in knowledge of the viruses of the potato-mosaic group it has become increasingly obvious that it is possible for one virus to produce different types of symptoms in different varieties, and that similar symptoms may be caused by different viruses. The disease, if any, produced by infecting a potato with a virus appears to depend almost as much on the variety of the host as on the virus. Failure to appreciate this has resulted in numerous names being given to the same virus, and similar names to different viruses, merely because different workers have used different varieties. In this paper the alphabetical system of nomenclature, instituted for potato viruses by Smith (1931), will be followed, and terms such as streak, mosaic, crinkle, etc., will be used only to describe the condition of infected plants.

Quanjer (1931) showed that the potato-streak diseases could be divided into two clinically distinct types, which he called "acropetal necrosis" and "top necrosis" (acronecrosis). Acropetal necrosis is the common leaf-drop streak of the field, and appears to be caused only by virus "Y", which Smith (1931) has shown to be transmitted by *Myzus persicae* Sulz. Virus "Y", however, does not cause this disease in all potato varieties but, in certain varieties, causes mosaic or crinkle symptoms, and it is possible that some of the differently named potato viruses which have been found to be aphis transmitted were really virus "Y".

Quanjer (1931) has defined top necrosis on the basis of the internal symptoms of affected plants as "Necrosis radiating from only a small percentage of the internal phloem strands, almost never from the external phloem strands, into the surrounding parenchyma, this in turn surrounded by a cork cambium, except in the tender tips which are soon killed:

occurring in foliage, stem and tuber." Externally the disease is seen as a necrotic spotting of the uppermost leaves, which is usually followed by the death of the growing points and the dying back of the plant from the top downwards. The extent and rapidity of the destruction of the plant vary somewhat with the age of the host and the environmental conditions (Bawden, 1932). Although top necrosis is rarely seen in the field it can be induced in different potato varieties by several viruses which are thought to be distinct, and such viruses are frequently to be found in the field in varieties in which they are carried or produce diseases less severe than top necrosis.

VIRUS "X"

Although no insect vector is known for potato virus "X" (Smith, 1931) it is the virus most frequently found in naturally infected potatoes, and few commercial stocks, other than those of the varieties Arran Crest, Epicure and King Edward, are free from it. The majority of varieties either carry virus "X" or, when infected, show interveinal mottles with but little dwarfing of the plant or deformation of the foliage. The varieties Arran Crest, Epicure and King Edward when infected either by needle inoculation or by grafting develop top necrosis and are killed. Plants in the second year of infection are acutely necrotic and soon die.

Salaman (1933) has shown that virus "X" occurs in strains varying in virulence, and that different strains may be present at the same time in infected plants. Samples of virus "X" have been obtained from a large number of potatoes, and these have given on tobacco (var. White Burley) symptoms varying from a barely perceptible mottle to a severe ringspot. When transmitted to President these all gave interveinal mottles, sometimes with slight necrotic spotting, which varied in intensity directly with the severity of symptoms on tobacco. In Arran Crest, Epicure and King Edward all the samples gave top necrosis. The American "healthy-potato virus" seems to be identical with virus "X". Samples of this which gave mottle and others which gave ringspot symptoms on tobacco, all gave top necrosis on Epicure and interveinal mottle on President. Also, they were found to react serologically with the serum of rabbits immunized against virus "X" (Spooner & Bawden, 1935).

VIRUS "D"

Virus "D" is closely related to virus "X" and should perhaps be regarded as an aberrant strain of it. Each virus reacts with antiserum prepared against the other, and plants infected with one acquire an immunity to further infection by the other (Bawden, 1934). Virus "D", however, differs from the various strains of "X" much more widely than these differ among themselves, and it has been named separately because of the distinct diseases that it causes in certain hosts. In tobacco and *Datura* virus "D" produces symptoms comparable with those produced by the mildest strains of "X", but in many potato varieties, it causes diseases more severe than those produced by the most virulent strains of "X".

The varieties Arran Crest, Epicure and King Edward react with top necrosis when infected with either virus "X" or "D". Virus "D", however, also causes top necrosis in Abundance, Arran Consul, Institut de Beauvais, Catriona, Duke of York, Dunbar Cavalier, Dunbar Yeoman, Majestic, Ninetyfold and Up-to-Date, all of which either carry virus "X" or show only interveinal mottles when infected with it. In President and certain other varieties virus "D" causes a characteristic necrotic disease, foliar necrosis, a detailed account of which has been published elsewhere (Bawden, 1934).

VIRUS "A"

Murphy & McKay (1932) described virus "A" which they found occurring alone in healthy-looking Irish Chieftain plants, and which they state is a necessary constituent of a virus complex causing the well-known disease crinkle. The other constituent Murphy & McKay called "simple mosaic", and it is apparently one or other of the strains of virus "X". Salaman (1930) showed that grafting from crinkled to healthy President plants reproduced the disease in full, whilst needle inoculation gave only an interveinal mottle. Later, Salaman (1932) showed that this mottle was due to virus "X". This has been confirmed many times and, in addition, successful syntheses of crinkle have always followed on the infection of apparently healthy Irish Chieftain plants with virus "X". Further, when "X"-infected mottled Presidents were grafted with scions from Irish Chieftain they became crinkled, whereas healthy Presidents similarly grafted either remained healthy in appearance or, at the most, developed only a fleeting mottle. The severity of the crinkling varied somewhat with the virulence of the strain of virus "X" used, but all sources of this virus when added to virus "A" gave crinkle symptoms in President.

Virus "A" alone in Arran Victory, President and the majority of potato varieties gives only a mild and fleeting mottle, or is carried. In Epicure the mottle is more definite and takes the form of a distinct yellowing around the veins. Virus "A" has been found occurring naturally in all the stocks of Irish Chieftain, Golden Wonder and Myatt's Ashleaf yet tested, whether these were healthy in appearance or not. Murphy & McKay (1932) showed that virus "A" produces a necrotic disease in Up-to-Date, and Clinch & Loughnane (1933) confirmed this and showed that it also produces top necrosis in British Queen. It has now been found that, in addition to these two varieties, virus "A" also causes top necrosis in International Kidney, Kerr's Pink, Rhoderick Dhu and Sharpe's Express. In one transmission only, top necrosis was also produced in Great Scot. The external symptoms differed slightly from those of the top necrosis produced by virus "X" in Epicure. The disease spread rather more slowly, and the individual necroses were fewer in number and larger. Internal necroses, however, were found arising in, and spreading from, the phloem in both stems and tubers. Plants in the second year of infection were small, brittle, highly necrotic and quickly died.

Clinch & Loughnane (1933) found that virus "A" was not transmitted to potatoes by needle inoculation, and this has been confirmed. It was suggested to the writer by Dr Dykstra of Corvallis, Oregon, U.S.A., that sap transmissions might be obtained by adding fine carborundum powder to the inoculum. This has been tried and found to be highly successful. When sap from President with crinkle was mixed with carborundum powder and rubbed on to the leaves of healthy President plants these developed full crinkle. Up-to-Date plants grafted with scions from such plants developed top necrosis, showing that virus "A" had been transmitted by this method of inoculation. Healthy President plants needle-inoculated with the same sap at the same time showed only an interveinal mottling and, when tested, were found to be infected with virus "X" alone. As yet, however, no successful infections of varieties in which virus "A" produces top necrosis have been obtained with the carborundum method, although twenty-five plants of Up-to-Date, Kerr's Pink and International Kidney have been inoculated.

Although virus "A" is not transmitted to potatoes by needle inoculation it is transmitted to tobacco by this method, as Clinch & Loughnane have shown (1933). Using this test attempts to determine the *in vitro* properties of virus "A" have been made. No infections were obtained with sap diluted more than 1/50, heated for 10 min. at 50° C. or higher, or from sap kept at room temperature for a day or more. These results, however, are probably not significant owing to the small percentage (average about 30 per cent.), of successful inoculations in the controls. It is hoped with the aid of carborundum powder to repeat these tests, and to get full infection with the controls.

Clinch & Loughnane also found that virus "A" was transmitted by *Myzus persicae* Sulz. This has been confirmed, but again the percentage of successful transmissions has been small. Of thirty-three attempted transmissions with *Myzus persicae* Sulz. only nine were successful, five of these being to tobacco and four to President. In tobacco (var. White Burley) the symptoms consisted of a mild vein clearing appearing a fortnight or so after infection, which faded and was followed by a definite dark green vein banding.

It has been suggested by Clinch & Loughnane (1933) that the viruses of the potato-mosaic group may be divided into an "X" and a "Y" group, and that virus "A" should be placed in the "Y" group. There would, however, seem to be little evidence in favour of this, for although viruses "A" and "Y" produce similar symptoms in tobacco and are both transmitted by *Myzus persicae*, they are certainly not related in the same way as are the various strains of virus "X". Tobacco plants infected with virus "A" were found to be quite susceptible to further infection with virus "Y". When inoculated with "Y" they showed a further vein clearing at the same time as healthy control plants of the same age, and further inoculations from such reinoculated tobacco plants to President resulted in the acropetal necrosis characteristic of infection with virus "Y".

VIRUS "B"

It is well known that individual plants of the variety Up-to-Date even when quite healthy in appearance are often virus infected, and that scions taken from them and grafted on to certain intolerant varieties cause top necrosis in the stocks. From work with many such carriers considerable evidence has now been obtained indicating that they are all infected with at least two viruses. The fact that different methods of transmission from these carriers to intolerant varieties gave different results first suggested this possibility. When scions were grafted to President stocks top necrosis followed, but needle inoculation of Up-to-Date sap to President never produced this disease. The results of needle inoculation have varied, and were sometimes nil. More often, however, the inoculated leaves developed black, circular local lesions. These remained discrete and did not spread, but were usually followed by systemic symptoms consisting of a general interveinal mottling, often accompanied by a certain amount of interveinal necrotic spotting.

Occasionally local lesions only were obtained, the uninoculated leaves remaining quite healthy. The young leaves of such plants when tested were always found to be virus-free.

When sap from the carrier Up-to-Date plants was needle inoculated to *Datura* or tobacco (var. White Burley) these developed symptoms characteristic of infection with virus "X". Seven to nine days after infection the veins of the young leaves became "cleared", and later there developed a general interveinal mottle with sometimes slight necrotic rings or spots. With sap expressed from different carriers the severity of these symptoms varied, indicating that they were infected with different strains, or mixtures of strains, of virus "X". It was further found that sap from inoculated President plants showing the mottle described above, when needled to tobacco and *Datura* produced symptoms identical with those following direct inoculation from the carrier. Immunity tests afforded further evidence that these symptoms were really due to the presence of virus "X". Tobacco and *Datura* showing these symptoms, whether infected direct from Up-to-Date or from the mottled Presidents, have always failed to develop any further symptoms when reinoculated with a virulent strain of virus "X", although control healthy plants of the same age developed severe ringspot. Also, expressed sap from inoculated President, tobacco and *Datura* plants (as well as the carriers) all reacted serologically with the serum of rabbits immunized with virus "X". It thus seemed quite conclusive that the carriers contained virus "X", which could be transmitted to potatoes, tobacco and *Datura* by needle inoculation. It was shown above that virus "X" causes only a mottle in President (the condition following inoculation with Up-to-Date sap), and in order to account for the production of top necrosis on grafting it was necessary to postulate the existence of a second virus in the carriers. This virus will be referred to as potato virus "B".

Clinch & Loughnane (1933) showed that the "Up-to-Date streak virus" was filterable through L 5 candles and transmissible to *Datura* by needle inoculation, for they found that scions taken from inoculated *Datura* caused top necrosis in grafted President stocks. This work has been repeated and fully confirmed, but it is difficult to agree with the conclusions of Clinch & Loughnane that the Up-to-Date carriers are infected with only one virus, and that the symptoms on *Datura* are due to this. The condition described by these workers is characteristic of infection with virus "X" and can be readily produced in *Datura* which will not cause top necrosis when grafted to President. As already stated, President inoculated from Up-to-Date developed only a mottle, but inoculations from this to *Datura* gave a result indistinguishable from that following direct inoculation from Up-to-Date. These *Datura* plants, however, never produced top necrosis in grafted President, whereas those inoculated direct from Up-to-Date did. Also, the results obtained by grafting or needling from these *Datura* plants were always the same, viz. an interveinal mottle with or without slight necroses, and there was nothing to indicate that they were infected with any virus other than "X". Thus a position was reached in which of two *Datura* plants showing identical symptoms one, when grafted to President, gave top necrosis but when needled gave only a mottle, whilst the other gave only a mottle whether grafted or needled. It seems, therefore, that virus "B" is transmissible to *Datura* by inoculation and that the first *Datura* plant was infected with both viruses "X" and "B", whilst the second was infected with "X" alone. As the symptoms in each were the same it is difficult to avoid the conclusion that they were due to the common factor, i.e. virus "X". Virus "B" has not yet been isolated and its reaction on *Datura* is not known, but from these results it seems probable that it would be carried.

Tomato, tobacco and *Nicotiana glutinosa*, as well as *Datura*, were infected with viruses "B" and "X" by needle inoculation. Inoculations of sap from Up-to-Date produced symptoms identical with those shown by the plants when they were infected with the same source of virus "X" alone. When scions were taken from them and grafted to President, however, top necrosis followed, indicating that they were infected with virus "B", in addition to "X".

Experiments with the progeny of President plants grafted with Up-to-Date carriers gave further evidence that the latter contain two viruses. Many of the tubers from plants with top necrosis became completely necrotic during storage and failed to grow. From those that grew three types of reaction were found. Some tubers gave minute plants, which were smothered with fine black necroses and quickly died. *Datura* inoculated from these developed an "X"-type mottle, and when grafted to healthy President caused top necrosis, indicating that viruses "X" and "B" were present. Other tubers gave plants which grew strongly and showed only a mottle and a little interveinal necrosis, a condition resembling that which Botjes (1934) has described as "attenuated top necrosis". *Datura* inoculated from these also developed an "X"-type mottle, but President plants grafted with such *Datura* showed only a mottle, indicating that they were infected with "X" alone. A certain number of tubers gave plants which grew strongly and looked healthy, and these, on testing, were always found to be virus-free. From this it seems that complete systemic infection does not necessarily occur in top necrosis, and that it is possible for the tubers of an affected plant to escape infection with either one or both viruses. The reason for this is not known, but it may lie in the killing action of the viruses, and in the fact that they become isolated in areas of dead tissue through which they are unable to spread.

Experiments with the local lesions obtained on inoculating President plants gave some indication that such a localization can occur. It was noted that local lesions were only formed when inoculations were made from Up-to-Date or other plants thought to be infected with both viruses "X" and "B", and not when made from plants infected with "X" alone. Thus President plants needled with Up-to-Date sap developed necrotic local lesions, and later a general mottle. Inoculations from the young mottled leaves to further healthy President plants gave the systemic mottling, but not the local lesions. This seemed to indicate that the lesions were probably due to virus "B", and the results of the following experiment supported this view. President plants were needled with Up-to-Date sap, and the necrotic local lesions were later punched out, ground up with a little water and inoculated to *Datura*. At the same time sap from the young mottled leaves was also inoculated to a set of *Datura* plants. Both sets of *Datura* developed identical symptoms ("X"-type mottling), but when scions from those inoculated with the local lesions were grafted to President they caused top necrosis, whilst grafts from the others caused only a mottle. From this it seems that both viruses "B" and "X" are transmitted by needle inoculation to President, but whereas the latter may spread throughout the whole plant the former does not, but is restricted to the necrotic tissue on the inoculated leaves. Virus "B" did not spread beyond the local lesions even in the inoculated leaves, for green areas punched out from between the necrotic ones contained only virus "X".

Arran Crest, Epicure and King Edward plants inoculated from Up-to-Date carriers developed top necrosis. Clinch & Loughnane (1933) also found this with Arran Crest and suggested that the streak virus becomes systemic in this variety when inoculated. The results of the following experiments, however, indicate that the top necrosis in these varieties following inoculation is due to virus "X" and not to virus "B". *Datura* inoculated from such plants with top necrosis developed an "X"-type mottle, and when grafted to President caused only a mottle in the stocks. Also, when reciprocal grafts were made between healthy

Epicure and President plants the same result followed, viz. mottle in the President and top necrosis in the Epicure portions, whether the inoculations were made to the Epicure or to the President parts of the grafted plants.

Virus "B" appears to be less resistant to dilution and ageing *in vitro* than virus "X". *Datura* inoculated with sap from plants infected with both viruses after being diluted 1 in 1000 or after 4 weeks' ageing became infected with virus "X" alone. The thermal death-point of the two, however, is similar. Plants inoculated with sap heated for 10 min. at 60° C. were infected with both viruses, whilst those inoculated with sap heated for 10 min. at 70° C. were not infected.

Immunity experiments have given further evidence that virus "B" is probably distinct from virus "X". Fifteen President plants infected with virus "X" were grafted with scions from carrier Up-to-Date plants. Seven of these developed typical top necrosis and were killed, and on three the scions grew poorly and no new symptoms appeared. The remaining plants showed many necrotic spots on the young leaves, and at first looked as if affected with top necrosis. The disease, however, spread extremely slowly, the necroses being restricted to the growing-point, and side shoots later appeared which grew normally and showed only a mottling. The reason for this localization of the symptoms in these plants is not known, and might perhaps be regarded as a type of protection. Later experiments, however, indicated that *Datura* plants infected with virus "X" are still susceptible to further infection with virus "B". Eighteen "X"-infected *Datura* plants were inoculated with sap from Up-to-Date, and none showed any change in symptoms. When scions from these reinoculated *Datura* plants were grafted to President, however, eleven caused top necrosis in the stocks, indicating that they had become infected with virus "B".

Plants of the following varieties have been found naturally infected with viruses "B" and "X": Arran Banner, Arran Consul, American Wonder, Burbank, Bliss Triumph, Duke of York, Earliest of All, Eclipse, Great Scot, Green Mountain, Majestic and Up-to-Date. When infected these all looked healthy or showed faint mottles characteristic of infection with "X" alone. Di Vernon, International Kidney and Myatt's Ashleaf can also act as carriers of these viruses, whilst Irish Chieftain shows crinkle when infected.

The following varieties are intolerant of viruses "B" and "X" and react to infection with top necrosis: Abundance, Arran Cairn, Arran Chief, Arran Comrade, Arran Crest, Arran Pilot, Arran Scout, Arran

Victory, British Queen, Catriona, Edzell Blue, Epicure, Golden Wonder, Katahdin, Kerr's Pink, King Edward, May Queen, President, Rhoderick Dhu and Sharpe's Express. As the writer has not obtained virus "B" free from virus "X" it is unknown whether the top necrosis in these varieties could be brought about by infection with "B" alone, or if it is a condition dependent on infection with both viruses. A recent publication by Dykstra (1935), however, seems to indicate that it might be caused by virus "B" alone. Dykstra has confirmed the finding that the carriers are infected with two viruses and, by grafting a potato seedling stated to be immune to virus "X" with scions from Up-to-Date, he has obtained a source of virus "B" free from "X". When transferred to Arran Victory and President this virus alone produced top necrosis.

VIRUS "C"

Salaman (1930*a*) showed that viruses other than those carried by the variety Up-to-Date could cause top necrosis, for he found that certain Di Vernon plants when grafted to Arran Victory and President gave top necrosis in the latter variety only, whereas grafting Up-to-Date scions gave top necrosis in both varieties. Further work with these carrier Di Vernon plants has confirmed this, and given additional evidence that they are infected with a virus other than virus "B". This virus which causes top necrosis in President and not in Arran Victory will be referred to as virus "C". Virus "C" has not been obtained alone. It has been found in two stocks of Di Vernon together with viruses "X" and "Y", and in one stock of Monocraat obtained from Prof. H. M. Quanjer together with virus "X".

Virus "C" has not been transmitted to any plants by needle inoculation. No local lesions were produced as a result of inoculation to President, and *Datura*, tobacco and tomato plants when inoculated and then grafted to President did not give top necrosis. Further differences between viruses "B" and "C" are seen in their reactions on various potato varieties. Virus "C" produces top necrosis in Arran Consul, Arran Banner, Duke of York, Eclipse, Majestic and Up-to-Date, all of which carry virus "B", as well as in Arran Crest, Arran Pilot, Arran Cairn, British Queen, Epicure, King Edward, President, and Sharpe's Express. It is believed to be carried by Arran Victory, Arran Chief, Great Scot and Rhoderick Dhu, for when plants of these varieties were grafted with Di Vernon they showed symptoms typical of infection with the other viruses present.

Summary

It is shown that top necrosis can be produced in different potato varieties by a number of viruses. The reactions of these viruses on a large number of commercial varieties are given, together with certain of their properties and methods by which they can be transmitted. By grafting and needle inoculating infected potatoes to the four varieties Epicure, Arran Victory, President and Up-to-Date, and noting the type of necrotic disease produced on these differential hosts, it has been found possible to distinguish with a fair degree of accuracy between six viruses. The necrotic reactions of these varieties are given below.

Virus	Needle inoculation to potato	Up-to-Date	Epicure	President	Arran Victory
"A"	–	Top necrosis	—	—	—
"B"	–	—	Top necrosis	Top necrosis	Top necrosis
"C"	–	Top necrosis	Top necrosis	Top necrosis	—
"D"	+	Top necrosis	Top necrosis	Foliar necrosis	Foliar necrosis
"X"	+	—	Top necrosis	—	—
"Y"	+	Acropetal necrosis	—	Acropetal necrosis	—

The writer has great pleasure in expressing his gratitude to Dr D. R. Salaman for permission to make use of the virus material, and for many valuable suggestions.

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THE SUSCEPTIBILITY OF THE PLANT CELL TO VIRUS DISEASE

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(With 1 Text-figure)

In connexion with some micromanipulative work which had been planned it became necessary to find a technique whereby the presence or absence of virus in a small amount of the contents of a cell of an infected plant could be demonstrated. This need led to several experiments bearing on the susceptibility of the host-plant cell, attempts being made to discover a plant, which, if suitably inoculated, would serve as an index for such minute amounts of virus. Unfortunately no plant and inoculation method yielded more than one-tenth of the expected number of infections. This could only be explained by a variation in the susceptibility of the cells of the host plants. It was decided to abandon these methods and to seek a serological test for such small quantities of virus. Nevertheless, the results of the experiments, although they were not statistically planned and data in some cases are scanty, are of sufficient interest to be worth placing briefly on record.

Inoculation without injury to the cells of the host

Experiments were first made to determine whether infection can occur when all the cells of the parts of a plant coming in contact with the virus are quite uninjured.

In the first series, three host plants which show systemic infection with the yellow strain of aucuba mosaic disease (2) were used. Juice was extracted from infected tomatoes, passed through fuller's earth and diluted with water to 1 part in 3. The upper and lower surfaces of healthy plants were sprayed with this juice through a de Vilbiss atomizer. Great care was taken to avoid touching the sprayed leaves in any way. In the control plants the upper surface of one leaf was similarly sprayed and was then rubbed with the finger.

The results accruing are shown in Table I.

Table I

Effect of spraying uninjured plants with suspension of aucuba mosaic virus

	Results			Controls		
Species	No. treated	+	−	No. treated	+	−
Solanum nodiflorum	36	0	36	12	12	0
Solanum lycopersicum	96	2*	94	24	24	0
Nicotiana tabacum	24	0	24	9	9	0

* Plants showed insect damage.

In a second series of experiments *Nicotiana glutinosa*, which develops local necrotic lesions on infection with tobacco or aucuba mosaic virus, was used. Whole leaves were sprayed with infectious juice. After spraying, one-half of each leaf was left untouched; as a control, the other half was rubbed with the finger to break the hairs which project from the leaf surface. Some leaves were treated on the plant; other leaves were detached from the plant and were minutely examined for any possible damage before spraying. After treatment the latter were stored in Petri dishes on damp filter paper. Care was taken to handle the leaves only by their petioles. Table II gives details of these experiments.

Table II

Effect of spraying uninjured leaves of Nicotiana glutinosa *with a virus suspension*

Inoculum	Position of leaf	No. of leaves treated	No. of lesions on sprayed halves	No. of lesions on halves sprayed and rubbed
Aucuba mosaic (yellow strain) of tomato, dilution: 1 in 100	On plant	14	3*	1167
Aucuba mosaic (yellow strain) of tomato, dilution: 1 in 100	Detached	15	0	1685
Tobacco mosaic (Johnson's No. 1), dilution: 1 in 10	On plant	18	5*	1417

* Showed insect damage.

Tables I and II show quite conclusively that the virus is unable to enter uninjured cells. In the very few cases where infection resulted after spraying, the leaves had been damaged by insects, presumably whilst the virus suspension remained on the leaf surface.

These results are in accordance with those of Caldwell (1), who holds that the virus cannot enter an unbroken cell. He states that, even when the virus is contained in the xylem vessels of the host, it is necessary to crush the tissues to enable it to pass from the xylem into the living cells.

Duggar & Johnson (3), however, state that it is possible to infect tobacco plants by spraying the leaves with infective juice, the virus passing into the host through the stomata. It is suggested that, in their experiments, "gently lifting the leaf with the nozzle of the atomizer and spraying while the leaf was so held" caused sufficient damage to the delicate hairs clothing the tobacco leaf for the virus to gain entry through a broken cell.

PERIOD WHICH MAY ELAPSE BETWEEN INJURY TO THE CELL AND THE ENTRY OF VIRUS

One-half of the adaxial surface of each of ten leaves of *Nicotiana glutinosa* was rubbed gently with the finger and immediately sprayed with a suspension of aucuba mosaic virus. The other half of each leaf was rubbed with the same virus suspension. The first method yielded 310 lesions; the second 329. It became evident that a high percentage of infection can be obtained if the virus is applied after injury is caused to the cells. Experiments were then made to determine how long after injury infection could still be brought about.

Leaves of *N. glutinosa* were rubbed gently with the index finger. One-half of each leaf so rubbed was immediately sprayed with a suspension of virus; the remaining halves were similarly sprayed after the lapse of a selected interval of time. In the first set of experiments intervals of 5, 10, 20 and 30 min. were selected. As it seemed desirable to obtain information regarding shorter periods, in the later experiments half-leaves were sprayed at intervals of 1, 2, 4, 8 and 16 min. after rubbing. Table III gives the results of these experiments, which are also graphically illustrated by Fig. 1.

Table III

Possibility of time elapse between injury to cell and entry of virus

Time interval in min.	No. of replicates	No. of lesions after interval	No. of lesions on controls	$\frac{\text{Lesions after delayed spraying}}{\text{Lesions after immediate spraying}}$ %
1	13	356	451	78·9
2	13	315	442	71·9
4	13	166	387	42·9
5	4	83	188	44·2
8	10	114	302	37·7
10	3	44	176	25·6
16	2	21	79	26·5
20	3	8	187	4·3
30	3	5	176	2·8

The results obtained do not accord with those of Holmes (4), who suggested that with *N. rustica* "rubbing is effective as an inoculation method only in the presence of virus", and that "wounds made immediately before the application of virus were ineffective". Fig. 1 shows that although the chances of infection fall rapidly in the first few minutes after injury, an adequate dose of virus may still gain entry into the cell even half an hour after rubbing.

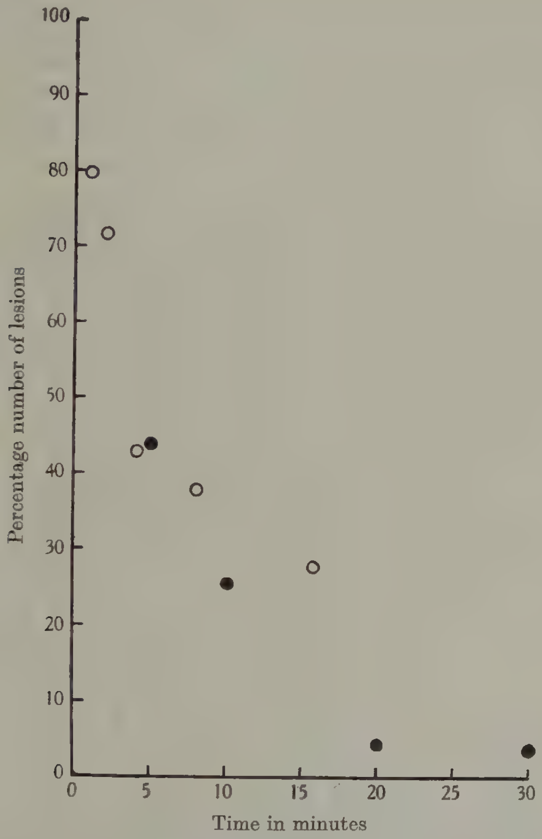

Fig. 1. The possibility of time elapse between injury to the cell and the entry of virus.

INOCULATION BY INJECTION INTO SINGLE CELLS

Doses of filtered but undiluted virus juice which should be adequate to cause infection were inoculated into single cells of growing plants.

Glass micropipettes of apertures 1–5μ were drawn out and suitably bent. A micropipette was fixed into the manipulating stand of a Janse and Péterfi micromanipulator bearing a microscope. A second microscope was placed beside the micromanipulator on the same side as the stand bearing the pipette and in such a position that the tip of the latter could be swung around, by adjusting the manipulating stand, from the optical axis of one microscope to that of the other. The second microscope was equipped with a glass stage on which was rested the leaf to be inoculated. To the objective of this microscope was attached a Busch diagonal illuminator. It was possible to pick up in the pipette a drop of virus juice from the cover-glass of the moist chamber and to inoculate it immediately into a cell of the leaf arranged beneath the second microscope.

Inoculations for systemic infection

Young plants of *Solanum nodiflorum*, tomato, tobacco and *Hyoscyamus niger* were inoculated with aucuba mosaic virus (yellow strain). One injection was made into each plant into the cells of either hairs, palisade or phloem tissues. No significant difference was observable between the results obtained by injection into different tissues. In all, 102 injections were made, nine infections resulting, i.e. a little less than 9 per cent. of the injections were effective.

Inoculations for necrotic lesions

Aucuba mosaic virus (yellow strain) was injected into the hairs or palisade cells of the leaves of *Nicotiana glutinosa*. The leaves were either detached from the plants and, after infection, stored in Petri dishes, or they were left on the plants which were returned to the glasshouse after inoculation. Leaves of that stage of development which experience suggested would give the greatest number of lesions were selected. Again the percentage of infection was low, and no significant difference was found between the percentage of infections resulting from injection into the hairs or into the palisade tissue. Of a total of 810 injections, 85 or 10·5 per cent. caused necrotic lesions to form on the leaves.

Similar injections were made into the cotyledons of the runner bean. Of these inoculations 15 in 240 or 6 per cent. resulted in the production of lesions.

Inoculations for starch lesions

The viruses of tobacco mosaic, aucuba mosaic and Hy. III diseases were injected into cells of hairs, palisade or phloem of tobacco leaves. A number of injections (usually fifteen) were made into one-half of each leaf; on the other half a standard dilution of virus juice was rubbed. The plants were returned to the glasshouse where they were left for a number of days varying according to the virus used and to the weather conditions. They were then put in total darkness and kept there from 6 p.m. to 9.30 a.m., when leaves were cut from the plant and immediately killed by immersion in boiling water. The leaves were decolorized with alcohol and stained in iodine solution and the numbers of lesions were counted.

The results obtained are summarized in Table IV.

Table IV

Inoculation of virus by micropipette into tissues of the tobacco plant

Tissue injected	Virus	No. of replicates	No. of starch lesions	Percentage of infections	Dilution of standard	Av. no. of lesions produced by standard
Hairs	Tobacco mosaic	42	6	14·3	1 in 100	115
Palisade	,, ,,	78	11	14·1	1 in 100	151
Hairs	Aucuba mosaic of tomato	162	8	4·9	1 in 10,000	26·4
Palisade	,, ,,	57	7	12·3	1 in 10,000	57·5
Phloem	,, ,,	105	19	17·1	1 in 10,000	75
,,	Hy. III	240	16	6·6	1 in 100	58·5

When the results were analysed, the figures obtained from the standard proved of little value. Dr M. A. Watson,[1] working in this laboratory, has similarly found the half-leaf method to be of little value when more than two variants are to be considered, and Youden & Beale (5) advocate the method when comparing only two samples of virus.

With tobacco mosaic no difference was found between the percentage of infections resulting from injection into the cells of the hairs and those of the palisade, but, with aucuba mosaic, inoculation into the palisade resulted in a higher percentage of infection. However, the χ^2 test shows this percentage not to be significantly greater. With aucuba mosaic, the percentage of infections obtained by inoculation into the phloem is found to be significantly greater than that obtained by inoculation of the hairs. Hy. III disease is normally transmitted by *Myzus persicae*, which is generally supposed to feed from the phloem of the host. For this reason, injections of Hy. III were confined to the phloem. The greater infection obtained by injection of aucuba mosaic than by Hy. III into the phloem may be explained by the greater infectivity of aucuba mosaic disease.

Inoculations by injection into single cells of various tissues of different hosts all yield low percentages of infection. This is not necessarily in disagreement with the results obtained by the more usual mechanical methods of inoculation. If, for instance, a leaf is rubbed with the finger or a cloth or spatula moistened with a virus suspension, a large number of hairs are torn or broken. To produce a systemic infection the virus needs to gain effective entrance through one only of these broken cells. If necrotic or starch lesions are shown, then the virus must enter the leaf at a number of cells. But the number of lesions produced is always exceedingly small as compared with the number of hairs on the leaf surface, and a considerable proportion of these may be broken by rubbing. The results accord with those of Dr M. A. Watson,[1] who finds that Hy. III virus is effectively carried by the aphid in only a small proportion of cases.

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[1] Unpublished data.

These low percentages of infection suggest that differences exist in the susceptibilities of cells to virus attack, not only in different leaves, but also in the same type of tissue and in the same leaf. It has been suggested that the varying susceptibilities of different leaves is due to some mechanical difference making tearing of the cells more difficult, and, for this reason, various abrasive substances have been mixed with the virus suspension. The fact that micropipette injections and infection by aphids yield low percentages of infection suggests that the varying susceptibility may be due to variation in some physical or chemical property rendering the cell contents antagonistic to the virus.

SUMMARY

A number of spraying experiments showed that the virus cannot enter a plant unless some of the cells are injured. It is not essential that such injury should be brought about in the presence of the virus. The chances of infection fall off rapidly in the first few minutes after injury, but infection occurs occasionally as long as half an hour after the cell is damaged.

Inoculations by micropipette into single cells of the host plant yielded only about one-tenth of the expected number of infections. This suggests differences in the susceptibility of the cells to virus attack.

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[1] Unpublished data.

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THE ROLE OF PLASMODESMS IN THE TRANSLOCATION OF VIRUS

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(With Plate XXI)

THE literature bearing on the movement of the virus within the plant has been summarized by Henderson Smith (3) and Kenneth Smith (4). The balance of the evidence suggests that virus is not carried in the transpiration stream, and the suggestion has been made that it travels in the phloem. That virus does travel more rapidly in the region of the vascular bundles is shown by the curious deformations of the usually circular starch lesions when near the larger veins.

It is obvious that on occasions the virus must move about in the parenchymatous ground tissue of the leaf. In the case of systemic infection the virus appears to be carried into the primary meristem of the shoot and there multiplies as the cells become differentiated. Interference with the normal development of the plastids results in the production of mottled leaves (2). But virus also finds its way into the older leaves which were fully developed when the plant was inoculated. If it is present in the palisade tissue of these leaves it must reach the cells by movement through ground tissue. Also when leaves are artificially inoculated by breaking of hairs in the presence of a virus suspension, the virus must then pass through the ground tissue. It has been suggested that the virus passes from cell to cell by "diffusion". Owing to the particulate nature of the etiological agent of these diseases, it is unlikely to pass from cell to cell by actual diffusion through the cell wall. It is suggested that it is carried along the protoplasmic strands which connect the cytoplasm of adjoining cells in the phloem and in the majority of other living tissues.

Over a period of years, the intracellular inclusion bodies induced by many viruses in their appropriate hosts have been examined. These inclusions occur to a greater or lesser extent in all tissues of the host. It was noticed that, although every cell over large areas of the epidermis might contain inclusion bodies, the guard cells of the stomata even when included in these regions were invariably devoid of such bodies (Pl. XXI, fig. 1). It was therefore decided to examine the protoplasmic connexions between these cells.

Epidermal strippings were taken from leaves of tomato, tobacco, *Hyoscyamus niger* and *Solanum nodiflorum*, all plants which had been used extensively in the study of intracellular inclusions. These were treated by Crafts's technique for demonstrating plasmodesms (1). After fixing, the material was immersed in sulphuric acid and was then stained. The immersion in acid causes the walls to swell and the plasmodesms to become stretched as the protoplast contracts from the walls. The strength of acid used and the time of immersion were varied. A shorter immersion in a weaker acid revealed the protoplasts of adjoining cells connected together by protoplasmic bridges (Pl. XXI, figs. 2 and 3). A longer immersion or a stronger acid caused these bridges to break at either end, a fine strand of cytoplasm remaining within the cell wall (Pl. XXI, fig. 4).

Preparations so made in no case revealed any protoplasmic connexions between the guard cells of the stomata and the surrounding epidermal cells. That these guard cells contained no intracellular inclusions (Pl. XXI, fig. 1) suggests that, owing to the absence of any direct protoplasmic connexion with the adjacent cells, the virus is unable to pass into the guard cells.

That the virus travels through the plasmodesms is a possible explanation of the non-transference of virus to the offspring of diseased plants even in cases where fruits and seeds are infective. The growing embryo generally absorbs nutriment through specially developed haustoria which ramify between the cells of the maternal tissue. The detailed structure and performance of these haustoria varies from species to species, but usually there seems to be no protoplasmic connexion between the young embryo and the tissues of the parent. If the virus were present in the embyro prior to fertilization, it would be expected to multiply rapidly in the meristematic tissue of the developing embryo. If it were not present prior to fertilization, unless it were brought by the pollen tube, it would be unable to reach the embryo later.

Any evidence as to the movement of virus within the host must of necessity be circumstantial. The plasmodesms constitute an obvious route for the passage of particulate substances from cell to cell of the parenchymatous ground tissue of the plant. That no evidence of the presence of virus could be found in cells having no protoplasmic connexion with virus-containing cells, suggests that the plasmodesms are indeed the path taken by the virus.

SUMMARY

Although intracellular inclusion bodies may occur in every cell over large areas of the epidermis, they have not been found in the guard cells of the stomata. No protoplasmic connexions could be shown to exist between the guard cells and the surrounding tissues. These findings suggest that, owing to the absence of plasmodesms, the virus is unable to reach the guard cells. Support is lent to the view that, when the virus moves in the ground tissue of the host, it is carried from cell to cell along the protoplasmic bridges.

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EXPLANATION OF PLATE XXI

Photomicrographs were taken with a Leitz "Makam" camera using a Leitz 6L objective and a Leitz 10 × periplanatic ocular. Magnification 450 ×.

Fig. 1. Epidermis of *Solanum nodiflorum* showing large intracellular inclusions. There are no inclusions visible in the guard cells of the stomata. (Fixed, Carnoy's fluid; stained, Feulgen and orange G.)

Fig. 2. Epidermis of tomato. Plasmodesms are shown between adjacent epidermal cells, but not between the guard cells and the epidermis. (Crafts's technique.)

Fig. 3. Epidermis of *Solanum nodiflorum.* Protoplasmic strands connect the cytoplasm of adjoining cells, but the guard cells are isolated. (Crafts's technique.)

Fig. 4. Epidermis of *Solanum nodiflorum.* Protoplasmic strands are seen in the swollen walls. (Crafts's technique.)

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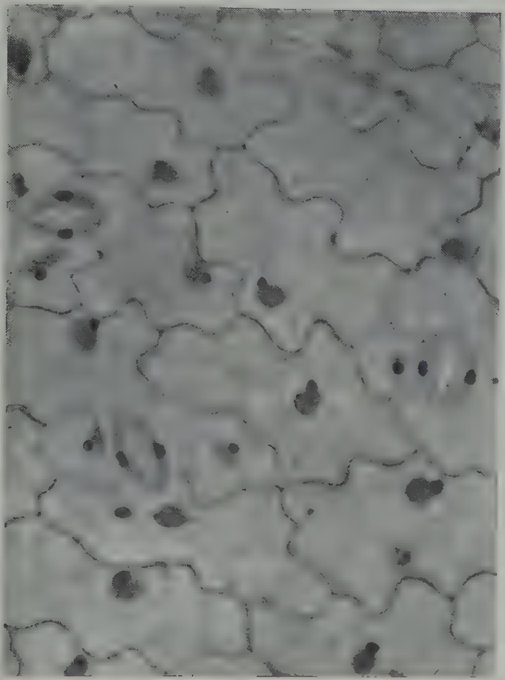

Fig. 1

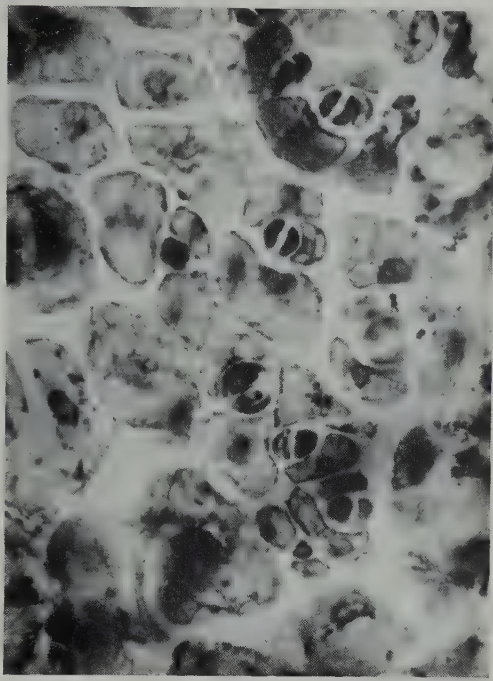

Fig. 2

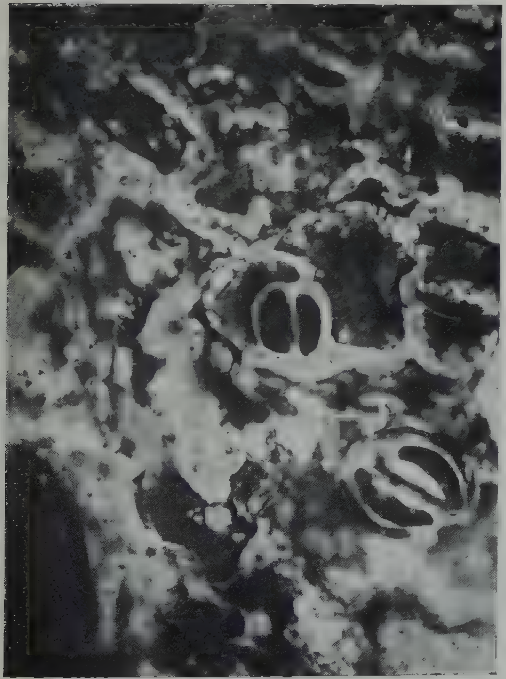

Fig. 3

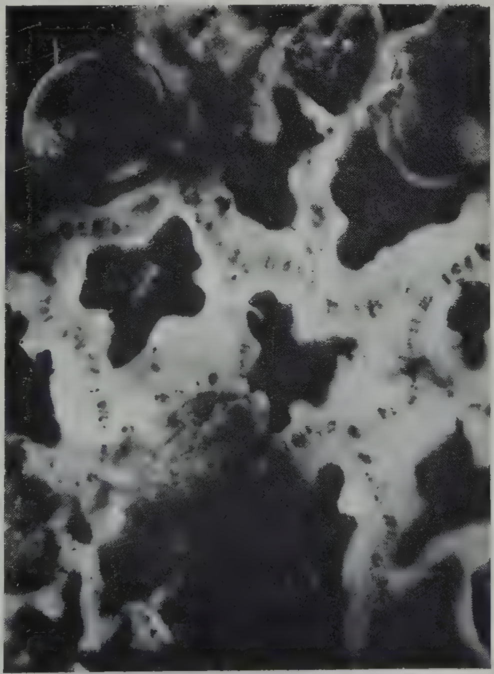

Fig. 4

SHEFFIELD—The Role of Plasmodesms in the Translocation of Virus (pp. 506–508)

THE REACTION OF THE VIRUSES OF TOMATO SPOTTED WILT AND TOBACCO MOSAIC TO THE *p*H VALUE OF MEDIA CONTAINING THEM[1]

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(With 6 Text-figures)

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I. Introduction

In studies on the nature and properties of any plant viruses *in vitro* it is essential to have some knowledge of the effects (if any) of the *p*H value of the suspension medium on virus activity. Such knowledge may also throw some light on the nature of the virus itself. In a previous paper (6) it was shown that the *p*H value of suspensions containing

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[1] Glasshouse facilities and part of the cost of these investigations were provided by the Council for Scientific and Industrial Research, Australia.

the virus of tomato spotted wilt (T. S. W.) affected the number of primary lesions produced when such suspensions were inoculated on to plants of *Nicotiana tabacum*. This work has now been carried further and has been extended to tobacco mosaic virus (Johnson's tobacco virus No. 1). In all our work use was made of the primary lesion method. The methods and technique as described in the previous paper (6) formed the basis of the work, and we have as a result been able to make direct intercomparisons and amongst other things to draw *p*H-activity and time-activity curves for the two plant viruses investigated.

II. Résumé of previous work

Prior to the completion of the work described in this paper the only work published on the effect of *p*H value on tomato spotted wilt virus was that mentioned above. With regard to tobacco mosaic virus Allard (1) had shown that this virus was resistant to acids and was inactivated by alkaline solutions, but no *p*H values were recorded. Brewer *et al.* (2) published some interesting observations on the effect of *p*H value on suspensions of tomato mosaic virus. These workers used the older method of systemic infection of whole plants (10–15 plants per treatment) as a means of estimating the activity of inocula, and the results can therefore be taken as qualitative only. Nevertheless they were sufficiently clear-cut to show that the virus activity of inocula in their experiments was reduced sufficiently between *p*H values of 7·5 and 8 to cause a falling off in the number of plants infected as compared with the untreated control inoculum. They further found that an inoculum inoculated at a *p*H value of about 8·5 had lost its infective power, but that when adjusted to lower *p*H values before inoculation it still remained infectious. They ascribed this behaviour to a reactivation of the virus. They also found that "the virus was not inactivated by an increase in acidity up to *p*H 2·46", but no data were presented on this point. Vinson & Petre (8) record critical values of about *p*H 2 and 9·2. Since the completion of the experiments described in this paper an excellent paper by Stanley (7) has appeared bearing on a section of our work with tobacco mosaic virus. Stanley's findings are discussed in the appropriate section of the present paper.

III. Experimental technique

(*a*) *Choice and arrangement of test plants*

The cultivation, selection and arrangement of test plants was similar to that previously outlined (6), extensive use being made of the Latin square combined with half-leaf comparisons. *N. tabacum* (var. Blue

Pryor) was the test plant used for tomato spotted wilt virus and *N. glutinosa* was used for tests with tobacco mosaic virus.

(*b*) *Source of virus*

For tomato spotted wilt virus tomato plants (var. Dwarf Champion) were artificially inoculated with the virus and were kept in the glasshouse in an isolated compartment. Leaves showing the typical bronze spots were crushed in a mortar and the juice was expressed by hand through fine book muslin. This juice was used without purification in the preparation of inocula because of the rapid inactivation of this virus *in vitro*. During the warmer summer months naturally infected field plants (Early Dwarf Red × Break o' Day hybrids) were used as source.

Tobacco mosaic virus was obtained from *N. tabacum* plants (var. Blue Pryor) artificially inoculated and kept in another isolated compartment of the glasshouse. Leaves showing symptoms of the disease were removed, crushed in a mortar and the juice was strained through book muslin. This juice was then exposed to air (but protected against outside contamination by a plug of cotton-wool) until the next day, when it was centrifuged at a force of about 1500 g. The supernatant liquid so obtained was pipetted off into a prepared container and was used as the stock from which the inocula were prepared. This stock was medium to dark brown in colour and showed only a faint turbidity.

(*c*) *Preparation of buffer solutions and determination of* pH *values*

In order to avoid any specific effects due to the use of different buffering substances a composite buffer was used throughout except where otherwise indicated in the text. A stock composite buffer solution was prepared which was 0·0533 M with respect to each of the following substances: boric acid, potassium dihydrogen phosphate and potassium hydrogen phthalate. In making up solutions this stock was treated with the appropriate amounts of 0·2 M HCl or 0·2 M NaOH and distilled water to give a final solution of the desired pH value and being 0·04 M with respect to total buffering substances. This stock buffer covered the whole range of pH values employed in this work and when diluted to 0·04 M strength had a pH value of about 4, so that the total electrolyte content of all buffer solutions prepared having a pH value at or above 4 was the same. The electrolyte content of solutions prepared by the addition of HCl to the stock would be higher by the amount of HCl added. The degree of dissociation of the various constituents, however, would vary with the pH value, so that the above arrangement represents the best practicable method of preparing a series of buffer solutions covering a wide range of *p*H values and having practically the same electrolyte content.

The *p*H values of inocula were determined in the first instance by both the hydrogen and quinhydrone electrodes, and when after many comparisons it was found that the two methods gave values within 0·01 *p*H unit of each other over the *p*H range in which the latter electrode is applicable, this was used between *p*H values of 2 and 8, and the bubbling hydrogen electrode was employed beyond these limits. In addition to the routine determination of the *p*H value of each inoculum shortly after preparation, determinations were made at intervals in those cases where aliquots of an inoculum were inoculated after various time intervals. In no case was there any marked change in the *p*H value of a given inoculum over the time ranges employed in this work. However, the addition of the juice to the buffer solution brought about an instantaneous change so that the *p*H value of the inoculum was not in general the same as that of the buffer solutions before the addition of the juice. The magnitude of this change varied, as might be expected, with the *p*H value of the buffer and the final concentration of juice, and was negligible over the *p*H range 5–8. The greatest change on the acid side of *p*H 7 was about 0·2 *p*H unit, and on the alkaline side the greatest changes occurred between 9·5 and 10·5, where the stock solution showed least buffering power. In this region the drop in *p*H value following the addition of the juice amounted to from 0·1 to 0·4 in extreme cases. For various reasons which will become clear after a consideration of the data presented later the *p*H value of the inoculum itself is considered in this work.

(*d*) *Preparation of inocula*

The suspension media were accurately measured out into suitable vessels, and aliquots of the stock virus suspensions prepared as described in section (*b*) were pipetted into them. In the case of tomato spotted wilt it was the usual practice for as many operators as there were inocula to pipette out juice simultaneously so that all inocula started off at potentially the same virus concentration.

To make up an inoculum with a concentration of infective juice of 1 in 200 (say), 1 ml. of juice was added to 199 ml. of the appropriate 0·04 *M* buffer solution. Since the juice itself contains considerable amounts of various salts the actual electrolyte content of the inocula would not be known unless an analysis of each sample of juice was made.

This, of course, is impracticable, but the electrolyte content of the inocula would differ from that of the buffer solutions by an exceedingly small amount which would be the same for all inocula prepared from the same stock sample of juice.

(e) *Technique of inoculation*

The technique of inoculation was essentially that described in a previous paper (6). The inoculum was applied by means of a ground-glass spatula and was washed off by a second operator immediately.

IV. INTERPRETATION OF THE LESION COUNT IN TERMS OF ACTIVE VIRUS UNITS

So far no method has been devised which will give even a reasonable estimate of the actual concentration of active virus particles in a given suspension. Dilution curves obtained for the viruses of ordinary tobacco mosaic ((4), (5), (9) and others) and for aucuba mosaic of tomato (3) have established that the primary lesion method is capable of giving a reasonably accurate estimate of *relative* concentrations provided the concentration of infective juice falls within a certain optimum zone where the relationship between dilution and number of lesions approximates to a straight line. There is some difference of opinion between the investigators concerned as to whether over this portion of the dilution curve a change in the relative concentration is accompanied by an equivalent change in the number of lesions or by a relatively smaller change. Such differences may be largely due to variations in the technique, but whatever the final verdict may be it is quite clear that we shall not be far out if we assume an equivalent change in the number of lesions consequent on any change in relative concentration of infective principle, keeping in mind the probability of an *under* estimation of any differences observed in a region corresponding to the flatter portions of the curve.

With regard to the virus of tomato spotted wilt no data bearing on the relationship between the concentration of infective juice and number of lesions is to be found in the literature, chiefly on account of the rapid inactivation of this virus *in vitro*. This difficulty has now been overcome by suspending the juice in a buffer solution at pH 7 containing sodium sulphite. The dilution curve so obtained is shown in Fig. 1. It will be seen that at high concentrations the number of lesions approaches a limit, but that below a concentration of about 1/120 a straight-line relationship holds. This curve represents the mean of two experiments carried out at the same time with the same inocula on the same plants (6 × 6 square of *N. tabacum*), one on the lower leaves and another on the upper leaves. At the higher concentrations the two experiments gave the same values. When the results of the two experiments are plotted separately and the straight-line portion of the curve is considered it is found that in the experiment corresponding to the lower leaves the change in relative concentration is accompanied by an equivalent change in the number of lesions, whereas for the upper leaves the reduction in the number of lesions is relatively greater than the corresponding reduction in the concentration of infective juice. The number of lesions produced at low concentrations is, however, small, and the experimental error is correspondingly high with a limited number of replicates. In plotting the curve we have therefore bulked the results of both experiments. Here again we shall not be greatly in error in interpreting the results of a particular chemical treatment if we work on the assumption that a change in the number of lesions is the result of an equivalent change in the concentration of active virus units provided always that any effect of the added chemical on the susceptibility of the host plant can be ruled out and that the initial concentration of juice in the control inoculum corresponds to a point on that portion of the curve over which this relationship holds.

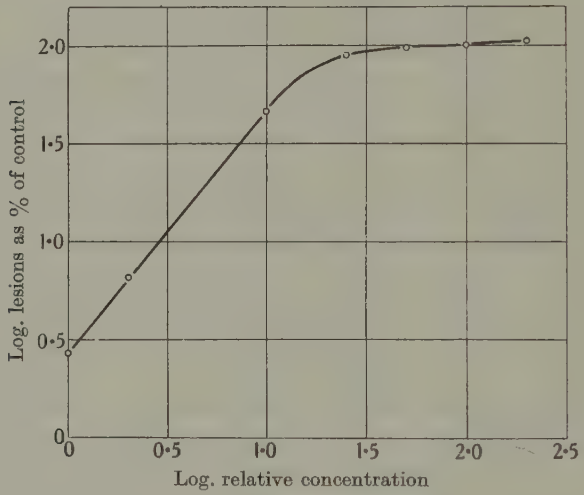

Fig. 1. Dilution curve for tomato spotted wilt virus buffered at *p*H 7 and in the presence of Na_2SO_3 (0·01 *M*), 21·5° C. Concentration range 1/15 to 1/3000.

In all our work with both viruses the concentration of juice was chosen so as to fall (as nearly as could be judged beforehand) on the straight-line portion of the dilution curve. Actually this condition may not always have been met, but short of performing dilution curves along with every experiment, this is the best that can be done. Further, the opposite half-leaf method of comparison was used throughout so that one-half of every leaf in any experiment was inoculated with a common control inoculum, the other half being inoculated with the appropriate test system. The half-leaves carrying the test systems were usually arranged in Latin squares. The ratio of the number of lesions obtained for any test system to the number obtained for the corresponding control will therefore give a number approximating to the relative concentration of this test system with respect to the control. Ratios derived in this way are used in this paper to represent relative concentrations of active virus units and are to be considered in conjunction with the limitations discussed earlier in this section.

V. THE ACTIVITY OF TOMATO SPOTTED WILT VIRUS AS AFFECTED BY THE *p*H VALUE OF SUSPENSIONS CONTAINING IT

As a result of previous experience with this virus, inocula were kept in an atmosphere free from oxygen up to the time of inoculation. This was done by passing a continuous stream of purified nitrogen through the inocula contained in vessels designed to exclude ingress of air. However, even under these conditions the concentration of active virus particles often fell off with time, presumably owing to the inactivating influence of some constituents of the juice added along with the virus. This feature is by no means constant. At room temperature the falling off may not begin for 24 hours, or it may become evident within an hour. It was found that maintaining the suspensions near 0° C. markedly retarded this anaerobic inactivation. Table I shows the relationship between virus activity and time for various *p*H values and 0° C. The figures for Exp. A represent the results of one experiment in which aliquots of the same stock of infective juice were used for the preparation of inocula, and those for Exp. B were obtained in an experiment carried out at a different time but under similar conditions.

Reference to Table I shows that even over the optimum *p*H range for this virus and at 0° C. inactivation may be quite marked. However, in many experiments of a similar nature suspensions of the virus at *p*H 7 and 0° C. in general maintained their activities unimpaired for 6 hours or more. As there does not appear to be a stable equilibrium

Table I

The change in activity of suspensions of tomato spotted wilt virus with time at pH *values of* 5·2, 6·0, 7·0, 8·7 *and* 9·2

Exp. A (control at *p*H 7·0). C=control at *p*H 7; T=test.

	Zero time		3 hr.		6 hr.		12 hr.	
*p*H	C	T	C	T	C	T	C	T
5·2	240*	30	235	0	227	0	120	0
6·0	281	247	303	108	199	36	90	1
8·7	260	272	331	351	181	241	85	163
9·2	260	160	237	205	238	158	94	93

* Numbers refer to total number of lesions on eight half-leaf replicates.

Exp. B.

*p*H	9 min.	36 min.	1 hr.	1 hr. 37 min.	2 hr.	4 hr.	6 hr.	10 hr. 52 min.
7*	397	412	403	492	485	473	421	401

* Portion of an experiment in which the control at *p*H 7 was inoculated on to an 8 × 8 Latin square at eight times as above. Each number refers to total number of lesions on sixteen half-leaf replicates.

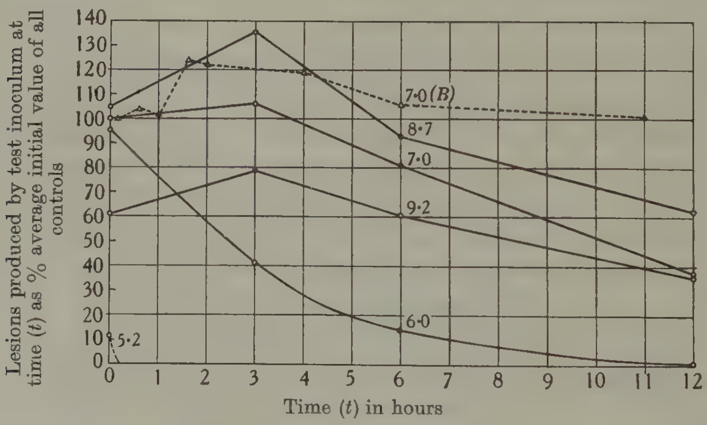

Fig. 2. Rate of inactivation of tomato spotted wilt virus at *p*H values of 5·2, 6·0, 7·0, 8·7 and 9·2. Temperature of inocula about 0° C.

for any *p*H value a *p*H-activity curve must relate to some specified time of contact between virus and medium. In all our work we have used a suspension buffered at *p*H 7 as a control inoculum, and as pointed out, such a suspension kept at 0° C. usually maintains its activity unimpaired for at least 6 hours. Comparisons over a wide *p*H range were therefore made as soon as possible after preparing the suspensions and again after an interval of 5 hours. Results of a typical experiment are summarized in Table II and Fig. 3.

Table II

Relationship between activity of suspension of tomato spotted wilt virus and pH *value*

*p*H value	4·3		5·1		6·1		8·0		8·9		9·4		10·6		12·1	
	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T
A. Mean inoculation time = 30 min. after adding juice to buffer solutions (cf. curve *A*, Fig. 3)																
No. of lesions on square on lower leaves	546	2	566	47	538	430	537	591	446	413	575	545	524	3	492	0
Ratio $T/C \times 10^2 = R_1$	0·37		8·3		79·9		110		92·6		94·8		0·57		0	
No. of lesions on square on upper leaves	507	0	592	86	663	655	758	669	564	574	607	482	625	4	660	0
Ratio $T/C \times 10^2 = R_2$	0		14·5		98·8		88·2		101·8		79·4		0·64		0	
Mean ratio $\frac{R_1 + R_2}{2}$	0·19		11·4		89·4		99·1		97·2		87·1		0·61		0	
B. Mean inoculation time = 5 hours after adding juice to buffer solutions (cf. curve *B*, Fig. 3)																
No. of lesions on square on lower leaves	557	1	681	0	572	663	621	515	619	626	629	516	625	0	649	0
Ratio $T/C \times 10^2$	0·18		0		115·9		82·9		101·1		82·0		0		0	
No. of lesions on square on upper leaves	693	0	822	0	760	914	840	863	969	946	751	624	861	0	775	0
Ratio $T/C \times 10^2$	0		0		120·3		102·7		98·2		83·1		0		0	
Mean ratio	0·09		0		118·1		92·8		99·7		82·6		0		0	
Mean of all ratios for both times (curve *C*)	0·14		5·7		103·8		96·0		98·5		84·9		0·31		0	

In the particular experiment to which Table II and Fig. 3 relate a uniform batch of 128 *N. tabacum* plants was arranged into two 8 × 8 squares, and 8 × 8 Latin squares were planned as described in a previous paper (6). Nine buffer solutions covering the *p*H range 4–12 and prepared as previously described were run into nine vessels (packed in ice) through which purified nitrogen was passing. After about 2 hours aliquots of the same sample of freshly expressed infective juice were run into these vessels simultaneously by nine operators. After allowing the contents of the vessels to become thoroughly mixed, samples were run into dishes packed in ice and the eight test systems were inoculated on to the two 8 × 8 Latin squares of the first sixty-four plants against the common control suspension at *p*H 7, using the half-leaf method. This particular *p*H value for the control suspension has been used throughout, since it falls within the optimum zone for this virus and in other respects has many advantages. Inoculations were made in order of plant number so that the mean inoculation time was the same for all systems. About 5 hours after the addition of the juice to the buffers this process was repeated with fresh portions of inocula on the two Latin squares of the second lot of sixty-four plants. Inocula were therefore exposed to air only during the inoculation process, and independent experiments have shown that at 0° C. inactivation of the virus is a relatively slow process even with a continuous air stream passing through the suspension.

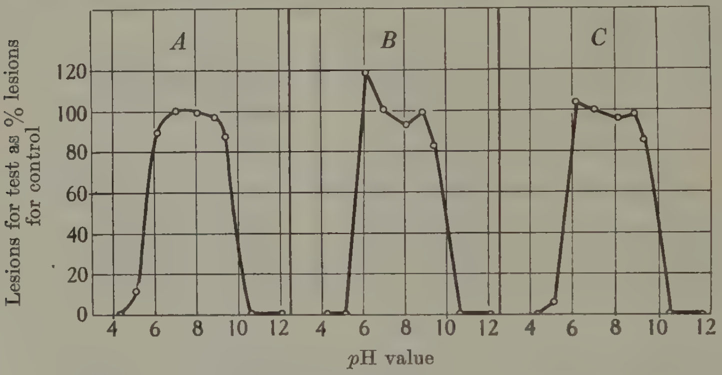

Fig. 3. Activity-*p*H curves for tomato spotted wilt virus in the absence of air and at 0° C. *A*, activity after 30-min. contact; *B*, activity after 5-hour contact; *C*, mean of *A* and *B*.

Since the common control suspension was inoculated on the one-half of every leaf in the square, the ratio of the number of lesions produced by each test system to that produced by the control has been used in constructing the curves of Fig. 3. On account of the necessity to reduce the handling of this virus to a minimum the inocula in these experiments were inoculated at the *p*H value of the suspension. However, special tests in which inocula at *p*H values of 5 were adjusted to *p*H 7 just prior to inoculation gave similar results. Other experiments in which the medium contained a reducing agent which maintained the reduction potential of the suspension well below the range at which oxidative inactivation is known to operate also indicated complete inactivation at *p*H values of 4 and 5, whether inoculated at the *p*H value of the suspension or after adjustment to *p*H 7. The critical *p*H ranges on both the acid and alkaline sides of *p*H 7 are the same when *N. glutinosa* is used as the test plant. In the case of tobacco mosaic virus many lesions are produced on *N. glutinosa* plants inoculated with suspensions as acid as *p*H 2. Further, in the case of tomato spotted wilt suspensions at *p*H 5, inoculation of the suspensions immediately after preparation results in the formation of many lesions per half-leaf, and the number decreases progressively with time until the last leaves of a series contain few or no lesions depending on the time taken to perform the whole operation. It would appear that the effects recorded are on the virus itself.

We may conclude therefore that the virus is rapidly inactivated at *p*H values at and below 5 and above a *p*H value in the neighbourhood of 10. The mechanism by which the inactivation occurs is not known particularly, as our suspensions contain constituents of the juice other than the virus. It is possible, for example, that on the acid side the action is in the nature of an irreversible precipitation of some constituents of the juice which carries the virus with it. In view of the fact that suspensions adjusted to *p*H 7 (after being subjected to lower *p*H, values) appear to be as highly dispersed as the control suspension this possibility may be ruled out for the present. Suspensions having a *p*H value above 7 are, if anything, more highly disperse than the controls, and the above possibility is again improbable. It appears, therefore, that the inactivation is connected with the action of hydrogen and hydroxyl ions on the virus particles. Studies along these lines may therefore give valuable information on those reactive groups of the virus concerned in these reactions, and this applies whether the virus is enzymic or organismal in nature.

A comparison of curves *A* and *B* of Fig. 3 in conjunction with the summary of analysis of variance (Table III) shows that these are similar in essentials and differ only in minor details, as, for example, in the activities at *p*H values of 6 and 9·4. In curve *A* there are no significant

Table III

Summary of analysis of variance of experiments recorded in Table II

pH value ...	4·3	5·1	6·1	8·0	8·9	9·4	10·6	12·1
A. 30-min. contact								
Mean diff. (C – T)	65·7	64·1	7·25	2·19	1·44	9·7	71·4	72·0

S.E. of each mean = 5·18; for diff. of 14·25 $P = 0{\cdot}01$ and for diff. of 12·7 $P = 0{\cdot}02$.

S.E. of difference between any two means = 7·32; for diff. of 20·1 $P = 0{\cdot}01$ and for diff. of 18·0 $P = 0{\cdot}02$.

B. 5-hour contact								
Mean diff. (C – T)	+78·1	+93·9	–15·3	+5·19	+1·00	+15·0	+92·9	+89·0

S.E. of each mean = 5·62; for diff. of 15·5 $P = 0{\cdot}01$ and for diff. of 13·8 $P = 0{\cdot}02$.

S.E. of difference between any two means = 7·95; for diff. of 21·9 $P = 0{\cdot}01$ and for diff. of 19·5 $P = 0{\cdot}02$.

differences between pH 6 and 9·4, whereas in curve *B* the difference between pH 6 and 9·4 is significant, when judged by Fisher's "t" test. However, in the majority of experiments where suspensions at pH values of 6 and 9·4 have been tested neither has differed significantly from the control at pH 7. In view of the many imperfections in even the best methods of estimating virus activity we shall postpone a consideration of such minor differences until a later date, when more information or better methods may make their interpretation less speculative.

It is of interest to note that the total number of lesions on the 128 half-leaves inoculated with the control suspension was 9200 at the first inoculation and 11,424 at the 5-hour inoculation. In view of the high count it is very likely that changes in the concentration of active virus units in this experiment have been under-estimated.

VI. THE EFFECT OF THE pH VALUE OF THE MEDIUM ON THE ACTIVITY OF SUSPENSIONS OF TOBACCO MOSAIC VIRUS *IN VITRO*

(*a*) *Time-activity relationships*

Time-activity curves at various pH values covering the range from pH 1 to 11 showed that the virus was very stable over the pH range 2–8, and that below a pH value of 2 and above a pH value of 9 partial or complete inactivation occurred. These curves further showed that when inactivation was only partial the greatest fall in activity took place within a few minutes of adding the juice to the buffer solution. This immediate rapid fall was followed by a further but slower fall with an approach to what appeared to be an equilibrium after a few hours. This reaction is shown for a buffer at pH *ca.* 9 in Figs. 4 and 5. The following account of the procedure adopted in arriving at Fig. 5, which illustrates the rate of inactivation of the virus in a buffer solution at *p*H 9, will serve as a general description of similar experiments.

Two stock virus suspensions were made up to double the virus concentration required for inoculation, one in the buffer solution under

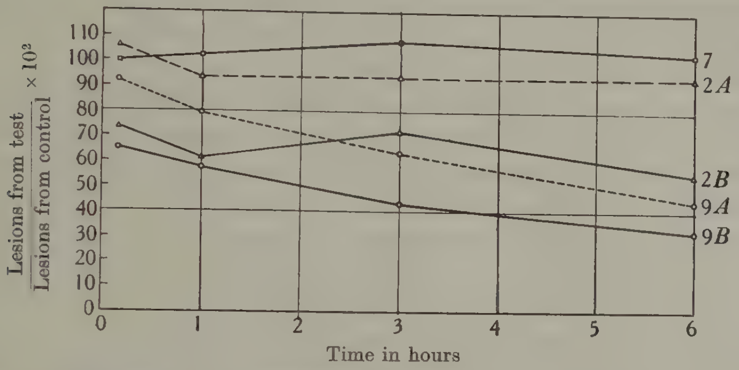

Fig. 4. Activity-time curves for suspensions of ordinary tobacco mosaic virus at *p*H values of 2, 7 and 9·4. Temperature of inocula 21·5 ± 0·5° C. *A*, adjusted to *p*H 7 before inoculation; *B*, unadjusted.

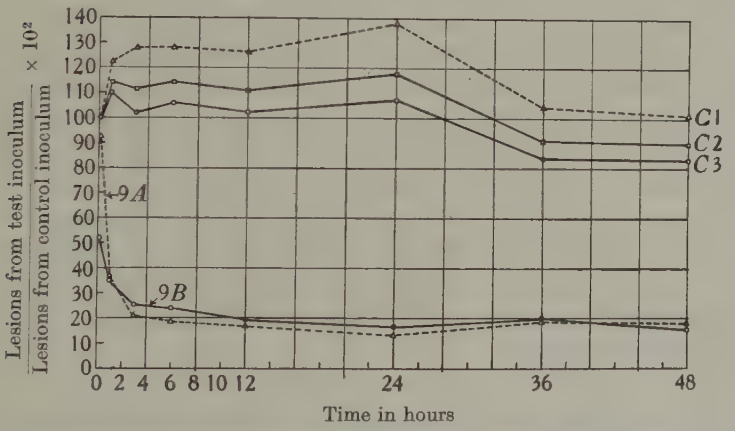

Fig. 5. Activity-time curves for suspensions of ordinary tobacco mosaic virus at *p*H values of 7 and 9. Temperature of inocula 21–23° C. *A*, adjusted to *p*H 7 before inoculation; *B*, unadjusted; *C*, controls at *p*H 7.

test (*p*H 9) and the other in a buffer solution at *p*H 7 (control). Portions of the same lot of centrifuged infective juice were used, so that the potential initial virus concentration was the same in each. The test stock was then divided into twice as many aliquots as there were to be time inoculations. Half of these were labelled as series A and half as series B.

An adjusting buffer solution had been prepared from the same stock composite solution as the test and control solutions and was 0·04 *M* with respect to total buffering salts, but was so adjusted that when added to an equal volume of the test buffer solution the *p*H value of the resulting solution would be the same as that of the control (viz. 7). At appropriate time intervals an equal volume of this adjusting buffer solution was added to an aliquot of the test suspensions of series A and an equal volume of the test buffer solution (*p*H 9) to an aliquot of series B. At the same time control inocula were prepared by adding an equal volume of buffer solution of *p*H 7 to the corresponding aliquots of the control suspensions. Actually the addition of adjusting buffer in series A took place when the inoculation of the corresponding suspension of series B was half-completed. Comparisons of relative virus concentrations were made by the half-leaf method. The test plants were sixty-four *N. glutinosa* plants trimmed to four leaves each and arranged in an 8 × 8 square. A separate Latin square had been planned for each set of leaves. The test suspensions of series A were inoculated against the common control on the opposite halves of the third and fourth leaves, while those of series B were similarly inoculated on to the first and second leaves. It will be seen that the test inocula of series A and the control inocula were identical in all respects but one, namely, that the virus of the test inoculum had been held at the *p*H value of the test suspension for the time specified, whereas the virus of the control inoculum had been held at *p*H 7 throughout. The test inocula of series B had been kept at *p*H 9 for approximately the same periods as those of series A but were inoculated at the *p*H value of the suspension (*p*H 9). In all cases inoculations were performed within a few minutes of the final addition of buffer solution. The concentration of infective juice in the inocula was 1 volume of juice in 200 volumes of inoculum (1/200). The *p*H value of each inoculum was determined just before or after inoculation.

The procedure for the experiment illustrated in Fig. 4 was on the same lines, except that in this case inoculations were made at four times only and the adjusted and unadjusted suspensions were treated as eight systems for the purpose of each 8 × 8 Latin square.

A summary of the actual lesion counts from which these curves have been constructed is given in Tables IV and V, and the analysis of variance is summarized in Table VI.

Table IV

Effect of suspending tobacco mosaic virus in buffer solutions of pH *values of* 9, 2 *and* 7 *for various time intervals on the number of lesions produced by subsequent inoculation on to* Nicotiana glutinosa *leaves*

C = control suspension. T = test suspension. Temp. of inocula 21·5 ± 0·5° C.

Time of contact ...	10 min.		1 hr.		3 hr.		6 hr.		10 min.		1 hr.		3 hr.		6 hr.	
	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T
	Test held at *p*H 9·4 and inoculated at *p*H 9·4. Curve 9*B*, Fig. 4								Test held at *p*H 9·4 and inoculated after adjusting to *p*H 7. Curve 9*A*, Fig. 4							
Square on first leaves	767*	526	963	534	933	430	943	323	742	703	761	645	735	444	758	307
Ratio T/C × $10^2 = R_1$	68·6		55·4		46·1		34·3		94·7		84·7		60·4		40·5	
Square on second leaves	705	437	698	363	806	320	693	203	766	688	715	528	761	505	659	311
Ratio T/C × $10^2 = R_2$	62·0		52·0		39·7		29·3		89·8		73·8		66·4		47·2	
Mean ratio $\frac{R_1 + R_2}{2}$	65·3		53·7		42·9		31·8		92·2		79·2		63·4		43·8	
	Test held at *p*H 2 and inoculated at *p*H 2. Curve 2*B*, Fig. 4								Test held at *p*H 2 and adjusted to *p*H 7 just before inoculation. Curve 2*A*, Fig. 4							
Square on third leaves	676	463	612	395	573	442	635	364	546	581	558	483	575	566	549	517
Ratio T/C × 10^2	68·5		64·5		77·1		57·3		106·4		86·6		98·4		94·2	
Square on fourth leaves	531	416	460	265	527	349	481	251	305	321	402	397	537	469	476	435
Ratio T/C × 10^2	78·3		57·6		66·2		52·2		105·2		98·8		87·3		91·4	
Mean ratio × 10^2	73·4		61·0		71·6		54·7		105·8		92·7		92·8		92·8	

* Each number not a ratio refers to the number of lesions on eight half-leaf replicates.

Table V

Effect of suspending tobacco mosaic virus in buffer solutions of pH *values of* 9 *and* 7 *for various time intervals on the number of lesions produced by inoculation on to* Nicotiana glutinosa *leaves*

C = control inoculum at *p*H 7. T = test inoculum. Temp. 21–23° C.

Time of contact ...	5 min.		1 hr.		3 hr.		6 hr.		12 hr.		24 hr.		36 hr.		48 hr.	
	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T
Test held at *p*H 9* and inoculated at *p*H 9 (series B). Curve 9*B*, Fig. 5																
Square on first leaves	290*	141	337	108	352	99	348	85	329	57	321	62	274	46	295	46
Ratio T/C × 10²	48·6		32·0		28·1		24·4		17·3		19·3		16·8		15·6	
Square on second leaves	302	168	314	120	252	58	280	65	277	60	312	56	224	52	200	32
Ratio T/C × 10²	55·6		38·2		23·0		23·2		21·7		17·9		23·2		16·0	
Mean ratio	52·1		35·1		25·6		23·8		19·5		18·6		20·0		15·8	
Test held at *p*H 9 and adjusted to *p*H 7 before inoculation (series A). Curve 9*A*, Fig. 5																
Square on third leaves	209*	199	203	75	271	77	264	50	243	35	210	25	214	49	196	36
Ratio T/C × 10²	95·2		36·9		28·4		18·9		14·4		11·9		22·9		18·4	
Square on fourth leaves	113	102	191	70	144	20	152	28	164	32	238	34	123	18	131	23
Ratio T/C × 10²	90·3		36·6		13·9		18·4		19·5		14·3		14·6		17·6	
Mean ratio T/C × 10²	92·7		36·7		21·1		18·6		16·9		13·1		18·7		18·0	

* Each number not a ratio refers to the number of lesions on eight half-leaf replicates, the mean ratio thus being derived from sixteen test and sixteen control replicates at each time.

Table VI

Summary of analysis of variance corresponding with Tables IV and V

A (cf. Table IV)

Time of contact ...	10 min.	1 hr.	3 hr.	6 hr.	10 min.	1 hr.	3 hr.	6 hr.
	Test held at *p*H 9·4 and inoculated at *p*H 9·4				Test held at *p*H 9·4 and inoculated at *p*H 7			
Mean diff. C – T	31·81	47·75	61·81	69·38	7·31	18·94	34·19	49·94

S.E. of each mean = 6·83; when diff. = 18·8 P = 0·01 and when diff. = 16·8 P = 0·02.
S.E. of diff. between any two means = 9·66; when diff. = 26·6 P = 0·01 and when diff. = 23·7 P = 0·02.

Time of contact ...	10 min.	1 hr.	3 hr.	6 hr.	10 min.	1 hr.	3 hr.	6 hr.
	Test held at *p*H 2 and inoculated at *p*H 2				Test held at *p*H 2 and inoculated at *p*H 7			
Mean diff. C – T	20·5	25·75	19·31	31·31	–3·19	5·00	4·81	4·56

S.E. of each mean = 4·40; when diff. = 12·1 P = 0·01 and when diff. = 10·8 P = 0·02.
S.E. of diff. between any two means = 6·22; when diff. = 17·1 P = 0·01 and when diff. = 15·3 P = 0·02.

B (cf. Table V)

Time of contact ...	5 min.	1 hr.	3 hr.	6 hr.	12 hr.	24 hr.	36 hr.	48 hr.
	Test held at *p*H 9 and inoculated at *p*H 9 (series B)							
Mean diff. C – T	17·69	26·44	27·94	29·88	30·56	32·19	25·00	26·06

S.E. of each mean = 2·92; when diff. = 8·03 P = 0·01 and when diff. = 7·2 P = 0·02.
S.E. of diff. between any two means = 4·13; when diff. = 11·4 P = 0·01 and when diff. = 10·1 P = 0·02.

Time of contact ...	5 min.	1 hr.	3 hr.	6 hr.	12 hr.	24 hr.	36 hr.	48 hr.
	Test held at *p*H 9·4 and adjusted to *p*H 7 just prior to inoculation (series A)							
Mean diff. C – T	1·31	15·56	19·88	21·13	21·25	24·31	16·88	16·75

S.E. of each mean = 2·07; when diff. = 5·7 P = 0·01 and when diff. = 5·1 P = 0·02.
S.E. of diff. between any two means = 2·93; when diff. = 8·1 P = 0·01 and when diff. = 7·2 P = 0·02.

(*b*) *Stability of suspensions at p*H 7

Previous tests have shown that provided the test plants had been transferred to the laboratory for at least 24 hours prior to inoculation their susceptibility does not change significantly when kept in the laboratory for further periods up to 4 days from the time of transfer. Any significant changes in the number of lesions produced by aliquots of a given inoculum when inoculated at intervals extending over this period may therefore be fairly interpreted as due to a change in the inoculum itself provided the conditions during and after inoculation are essentially the same. These conditions have been met in the experiments described in this paper, with the exception of minor details of an uncontrollable nature which will be discussed as occasion arises.

It is therefore interesting to note that in the experiments just described the number of lesions given by the control inoculum does not change significantly over a period of at least 24 hours. Curve 7 of Fig. 4 (cf. Table VII) shows that the control inoculum remained constant over the 6-hour period of this experiment. Each point on this curve is derived from sixty-four half-leaf replicates (sixty-four different leaves), and represents the total number of lesions on the control half-leaves at any time, expressed as a percentage of the number at the first inoculation. The curves C_1, C_2 and C_3 of Fig. 5 are arrived at in the same way but are based on fewer replicates (16, 16 and 32 respectively per point) and show greater deviations, although considering the limitations of the biological method and the fact that the inoculations were spread out over 48 hours thereby introducing a much larger personal factor, the departure of these curves from the horizontal straight line is not great.

Table VII

Summary of analysis of variance of controls only *of the experiments tabulated in Table IV* (*cf. Fig.* 4)

Time	10 min.	1 hr.	3 hr.	6 hr.
Mean no. of lesions per half-leaf for 32 replicates of controls on first and second leaves (corresponding with *p*H 9·4 series)	93·1	98·0	101·1	95·4
S.E. of each mean = 5·99. S.E. of diff. between any two means = 8·47, therefore for diff. of 23·3 $P = 0{\cdot}01$, and for diff. of 20·8 $P = 0{\cdot}02$.				
Mean no. of lesions per half-leaf for 32 replicates of controls on third and fourth leaves (corresponding with *p*H 2 series)	64·3	63·5	69·1	66·9
S.E. of each mean = 4·71. S.E. of diff. between any two means = 6·66, therefore for diff. of 18·3 $P = 0{\cdot}01$, and for diff. of 16·4 $P = 0{\cdot}02$.				
Mean no. of lesions per half-leaf for 64 replicates of controls on all four sets of leaves	78·7	80·8	85·1	81·2
S.E. of each mean = 3·79. S.E. of diff. between any two means = 5·36, therefore for diff. of 14·7 $P = 0{\cdot}01$, and for diff. of 13·2 $P = 0{\cdot}02$.				

Reference to Table VIII shows that taking curves C_1 and C_2 separately (A and B of Table VIII) there is no significant change over the 48-hour period ($P < 0{\cdot}02$ throughout). In both C_1 and C_2 there is, however, a drop from the 24-hour to the 36-hour inoculation. The 48-hour inoculation shows no further change, but the lower numbers are sustained. Combining the results of both experiments (they were done on the same plants) we see from Table VIII C that this drop is just significant at the $P = 0{\cdot}02$ level. Since the 48-hour figures are practically identical with the 36-hour ones, this fall in the number of lesions between the sixth and seventh inoculations is most probably due to a change in the susceptibility of the test plants.

Table VIII

Summary of analysis of variance of controls only *of the experiment tabulated in Table V* (*cf. Fig.* 5)

B. Mean no. of lesions per half-leaf for 16 replicates of controls on first and second leaves (corresponding to series B of Tables V and VI) (cf. curve C_2, Fig. 5)

Time ...	5 min.	1 hr.	3 hr.	6 hr.	12 hr.	24 hr.	36 hr.	48 hr.
B	37·0	40·7	37·75	39·25	37·9	39·6	31·1	30·9

S.E. of each mean = 3·68.

S.E. of diff. between any two means = 5·20, therefore for diff. of 14·3 $P = 0{\cdot}01$, and for diff. of 12·8 $P = 0{\cdot}02$.

A. Mean no. of lesions per half-leaf for 16 replicates of controls on third and fourth leaves (corresponding to series A of Tables V and VI) (cf. curve C_1, Fig. 5)

Time ...	5 min.	1 hr.	3 hr.	6 hr.	12 hr.	24 hr.	36 hr.	48 hr.
A	20·1	24·6	25·9	26·0	25·4	28·0	21·1	20·4

S.E. of each mean = 2·37.

S.E. of diff. between any two means = 3·35, therefore for diff. of 9·21 $P = 0{\cdot}01$, and for diff. of 8·23 $P = 0{\cdot}02$.

C. Mean no. of lesions per half-leaf for 32 replicates of controls for all leaves (cf. curve C_3, Fig. 5)

Time ...	5 min.	1 hr.	3 hr.	6 hr.	12 hr.	24 hr.	36 hr.	48 hr.
C	28·6	32·7	31·85	32·6	31·7	33·8	26·1	25·7

S.E. of each mean = 2·16.

S.E. of diff. between any two means = 3·05, therefore for diff. of 8·39 $P = 0{\cdot}01$, and for diff. of 7·5 $P = 0{\cdot}02$.

(*c*) *Virus activity at* pH 2

From Fig. 4 and Tables IV and VI A it is clear that when suspended in a buffer solution of *p*H 2 and inoculated at this *p*H value significantly fewer lesions result than from the control suspension. The drop takes place immediately, and no significant changes take place from the 10-min. to the 6-hour inoculation. On the other hand, when the suspensions held at *p*H 2 are adjusted to *p*H 7 just before inoculation so as to eliminate any effect on the test plant due to differences in the *p*H value of the inocula, the number of lesions is not significantly different from that of the controls at any one time and there is no significant change over the 6-hour period, although the counts for the 1-hour, 3-hour and 6-hour inoculations all lie below those for the corresponding controls by 7·3, 7·2 and 7·2 per cent. respectively.

An interesting problem now presents itself in finding an explanation for the large difference between the adjusted and unadjusted suspensions which is clearly significant. There are two possible explanations. (1) There is no difference in the actual virus concentrations of suspensions in the two series, but inoculation at *p*H 2 lowers the susceptibility[1] of the test leaves so that fewer lesions result. (2) The differences observed reflect real differences in virus concentration, smaller differences obtained in the adjusted suspensions being due to a reactivation of previously inactivated virus particles.

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[1] This effect may be a complex one. For example, the *p*H value of the inoculum may determine the state of the protoplasm at the point of entry of the virus into the leaf and so make entry more difficult in some cases, and further, since some of the medium will

Either of the effects outlined in (1) and (2) would suffice to explain the facts or both effects may operate. In view of the probable effect on the susceptibility of the test leaves we cannot arrive at any conclusions concerning a reactivation in this instance, although we cannot ignore the possibility, especially in view of the fact that on the alkaline side (at *p*H 9 for example) a partial reactivation is indicated beyond reasonable doubt.

(*d*) *Virus activity at p*H 9·4

Fig. 4 and Tables IV and VI A show that at *p*H 9·4 a significant rapid drop in virus activity took place during the first 10 min., followed by a further slow but steady inactivation which had not reached an end-point at the conclusion of the experiment. In this respect both the adjusted and unadjusted suspensions behaved alike. Further, the experiment (carried out some weeks later) illustrated by Fig. 5 and Tables V and VI B clearly shows that there is no differential effect on the susceptibility of leaves inoculated with suspensions at *p*H values of 7 and 9. We may therefore be confident that in this case the differences in the number of lesions represent real differences in the virus concentration.

The downward trend of both curves (9*A* and 9*B* of Fig. 4) is significant. An interesting feature is that the difference between the adjusted and unadjusted suspensions gets progressively smaller with time as seen by the gradual approach of the curves 9*A* and 9*B*. The differences between the two series at any time are significant except at the last inoculation (see Table VI), and the diminishing difference, i.e. the approach of the curves, is also significant. The ratio of the amount of active virus in inocula of series B at any one time to that in the corresponding inocula of series A appears to be a constant, having the values 0·71, 0·68, 0·68 and 0·73 at the first, second, third and fourth inoculations respectively. We may therefore conclude that portion of the virus was completely and irreversibly inactivated at *p*H 9·4, that the amount of

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enter along with the virus the presence of a solution buffered at a *p*H value very different from that normal to the part of the leaf where multiplication of the virus takes place may affect the metabolic processes in such a way as to make multiplication of the virus more difficult. *These and other possible effects are all grouped together under the term "susceptibility" for the sake of simplicity.*

virus so inactivated increased with time, that a small portion was reversibly inactivated (i.e. was reactivated when suspensions were changed from *p*H 9·4 to 7), and that the amount capable of reactivation decreased progressively with time during the 6-hour period of this experiment.

(*e*) *Virus activity at p*H 9

As a result of the above findings a further experiment was carried out in order to check the reactivation effects, and further, to see whether the downward trend would continue to the point of complete irreversible inactivation or whether an equilibrium state would be reached. When the difference between corresponding points on curves 9*A* and 9*B* was plotted against the time and the curve was extrapolated to the time axis it cut this at about the 10-hour point. The new experiment was therefore planned to cover a period of 48 hours with the greater number of inoculations falling within the first 12 hours. From Fig. 5 and Tables V and VI B it will be seen that the large immediate drop and the reactivation effect (both significant) are confirmed. In this experiment, however, the reactivation effect is confined to the first inoculation, and subsequent to this there is no significant difference between adjusted and unadjusted suspensions at any one time. The virus activity in both series falls for the first few hours and thereafter remains constant, there being no significant difference between the lesion counts for either series over the last 45 hours. Taking the average of the four counts covering the last 36-hour period, we find that for the adjusted series the lesions have been reduced to 16·7 per cent. of the corresponding controls and for the unadjusted series to 18·5 per cent. As pointed out previously, this circumstance makes it very unlikely that there has been an effect on leaf susceptibility of inoculating at *p*H 9 as compared with *p*H 7. The possibility that a depressing effect at *p*H 9 on leaf susceptibility would be just counterbalanced by a reactivating effect on the virus at all four inoculation times is so negligible that it may be ruled out, particularly when taken in conjunction with the previous experiment. The attainment of what appears to be an equilibrium state is very interesting and is discussed later.

(*f*) *p*H *activity curve for tobacco mosaic virus*

In view of the fact that the infectivity of inocula buffered at the critical *p*H range appeared to reach a steady state in something less than 12 hours, this time of contact between virus and buffer was chosen in making a comparison of the effect of *p*H value on the activity of the virus. A number of buffer solutions was prepared to cover the pH range from 1 to 11, and suspensions of the virus (from a common stock) were prepared and allowed to stand at room temperature (19° C.) for 12 hours. Each suspension was then divided into two portions, to one of which was added an equal volume of the original buffer solution used in its preparation, and to the other portion an equal volume of adjusting buffer was added as previously described. The two series of suspensions were then inoculated on to the leaves of a 10 × 10 square of *N. glutinosa* plants. The suspensions of the unadjusted series (B) were inoculated on to the first and second leaves arranged in two independent 10 × 10 Latin squares, and the suspensions of the series adjusted to pH 7 (A) were inoculated on to the third and fourth leaves of the same plants arranged

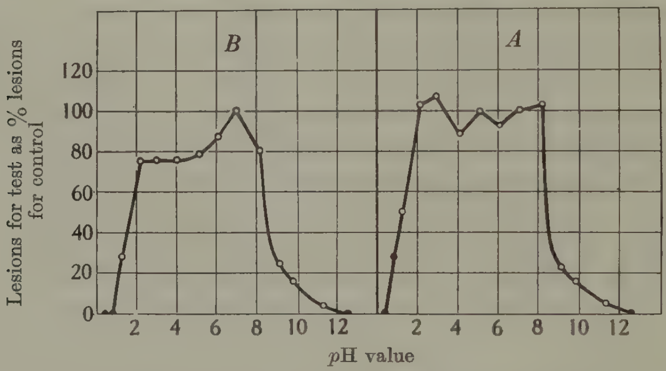

Fig. 6. Activity-pH curves for ordinary tobacco mosaic virus. 12-hour contact at 18° C. *A*, suspensions adjusted to pH 7 before inoculation; *B*, suspensions not adjusted.

in two further independent Latin squares. In both series the virus had been in contact with the buffer at the indicated pH value for 12 hours. They differed, however, in that series B was inoculated at the test pH value, whereas series A was inoculated after first adjusting the test suspensions to pH 7, the pH value of the common control inoculum. Comparisons were made as usual by the half-leaf method, so that one-half of every leaf (400 in all) was inoculated with the same control suspension which had remained at pH 7 throughout. The concentration of all inocula with respect to infective juice was 1/200.

The results of this experiment are set out in Fig. 6 and Tables IX and X. Previous to this experiment a number of similar but smaller experiments had been carried out in which the time of contact was shorter and where narrower pH ranges were covered per experiment. These experiments gave results in full accord with the one described,

Table IX

Effect of pH *value on the activity of tobacco mosaic virus* in vitro

*p*H value ...	1·18		2·14		2·97		4·04		5·07		6·09		8·23		9·09		9·77		11·27	
	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T
A. Suspensions kept at *p*H values indicated for 12 hours and then adjusted to *p*H 7 just prior to inoculation. Common control suspensions at *p*H 7·12																				
Square on third leaves	344	183	267	292	352	331	253	281	273	206	324	295	339	374	245	54	193	31	319	16
Ratio T/C × $10^2 = R_3$	53·2		108·5		94·0		111·1		75·5		91·0		110·3		22·05		16·1		5·02	
Square on fourth leaves	142	67	226	217	235	283	247	160	273	334	304	290	312	293	187	42	289	40	301	11
Ratio T/C × $10^2 = R_4$	47·2		96·0		120·4		64·8		122·3		95·4		94·2		22·5		13·8		3·65	
Mean ratio $\frac{R_3+R_4}{2}$	50		102		107		88		99		93		102		22·3		15·0		4·3	
B. Suspensions kept at *p*H value indicated for 12 hours and inoculated at this *p*H value. Common control same as for A																				
Square on first leaves	328	110	258	199	304	232	247	169	326	202	237	199	263	222	251	63	302	57	259	9
Ratio T/C × $10^2 = R_1$	33·5		77·1		76·3		68·5		62·0		84·0		84·4		25·1		18·9		3·47	
Square on second leaves	331	77	290	211	390	296	300	245	304	289	248	222	353	274	328	76	350	45	323	8
Ratio T/C × $10^2 = R_2$	23·2		72·7		75·9		81·7		95·0		89·5		77·6		23·2		12·9		2·48	
Mean ratio $\frac{R_1+R_2}{2}$	28·4		75		76		75		78·5		87		81		24·1		15·9		3·0	

C. Suspensions in 0·5 *N* HCl (*p*H 0·35) and in a buffer solution at *p*H 12·47 were completely inactive after 15-min. contact. No lesions were produced on the sides inoculated with the test suspensions (adjusted to *p*H 7) whereas many lesions were produced by the control inocula.

Table X

Summary of analysis of variance of results of the experiments tabulated in Table IX

*p*H value ...	1·18	2·14	2·97	4·04	5·07	6·09	8·23	9·09	9·77	11·27
A. Test held at above *p*H value and inoculated at *p*H 7										
Mean difference C − T, 20 replicates	+11·8	−0·8	−1·35	+2·95	+0·3	+2·15	−0·8	+16·8	+20·55	+29·65

S.E. of each mean = 2·75; when diff. = 7·56 $P=0·01$ and when diff. = 6·76 $P=0·02$.
S.E. of diff. between any two means = 3·89; when diff. = 10·7 $P=0·01$ and when diff. = 9·56 $P=0·02$.

*p*H value ...	1·18	2·14	2·97	4·04	5·07	6·09	8·23	9·09	9·77	11·27
B. Test held at above *p*H value and inoculated without adjustment										
Mean difference C − T, 20 replicates	23·6	6·9	8·3	6·65	6·95	3·2	6·0	22·0	27·5	28·25

S.E. of each mean = 2·44; when diff. = 6·71 $P=0·01$ and when diff. = 6·0 $P=0·02$.
S.E. of diff. between any two means = 3·45; when diff. = 9·49 $P=0·01$ and when diff. = 8·48 $P=0·02$.

but since the present experiment was more carefully controlled, being, in fact, based on the results of previous experiments, we shall omit the details and results of these earlier experiments except where they supplement the present one, such as for *p*H values above 11 and below 1.

Reference to curve *A* (Fig. 6) and Tables IX A and X A shows that there has been no significant change in virus activity under the conditions of these experiments over the *p*H range 2–8·2. On the acid side of *p*H 2 a significant drop in activity has taken place which becomes greater with increasing acidity until in a 0·5 *N* HCl solution inactivation is complete and irreversible. On account of the alkalinity of the adjusting buffers used to adjust such a highly acid solution to *p*H 7 there was a possibility that a fraction of still active virus would be inactivated by local regions of high *p*H value produced during the addition, even though the likelihood of such conditions developing was minimized by vigorous stirring whilst adding the adjusting solution. Tests were therefore carried out in which the preliminary adjustment was made by adding solid $CaCO_3$ to the acid suspensions until the *p*H value was brought to 5 or 6 and then adding an equal volume of buffer solution at *p*H 7. Where lesions resulted (in the case of *p*H 1, for example) there was no significant difference between the suspensions adjusted by means of the alkaline buffer solution, and the corresponding aliquot adjusted by means of $CaCO_3$ provided the slight abrasive action of the excess $CaCO_3$ was eliminated by adding some solid $CaCO_3$ to the other inoculum. In the case of 0·5 *N* HCl suspensions in order to reduce the electrolyte content of the inoculum the virus was allowed to remain in contact with the acid at a concentration of 1 volume juice in 20 of suspension, finally diluting to 10 times this volume before inoculation. The electrolyte concentration of the final inoculum was therefore not very different from that of the inoculum prepared by adjusting to *p*H 7 with buffer solution. The two inocula were inoculated against each other on opposite halves of ten leaves, and no lesions resulted in either case. A control inoculum prepared from the same stock juice but maintained at *p*H 7 throughout was inoculated on the bottom leaves of the same plants and gave an average of 16·5 lesions per half-leaf. Since the two methods used to adjust acid suspensions to *p*H 7 give the same results, and since the $CaCO_3$ is known to have no effect on the virus it is clear that there can have been no inactivation due to the use of alkaline adjusting buffer solutions.

Above *p*H 8·2 a sharp, significant fall in activity occurs until at *p*H 12·5 inactivation is complete. It will be observed that there is no significant difference between corresponding pairs of points on curves *A* and *B* for *p*H values of 7 and above. At *p*H values below 2 the adjusted suspensions are significantly higher than the unadjusted ones except where complete inactivation has occurred. The shape of this portion of both curves is the same, apparently a straight line of steep slope.

The curves differ in the section between *p*H 2 and 7, that for curve *B* showing a gradual but distinct falling off in the number of lesions produced relative to the control. At *p*H 6 the mean difference from the control is 3·2 with a S.E. of 2·44 and obviously not significant, but at *p*H values of 2, 3, 4 and 5 the difference from the corresponding control is significant with values for *P* between 0·02 and 0·01. The same argument as was used in discussing the time-activity curve at *p*H 2 (p. 527) may be applied to the points on this portion of the curve. It may be added that there is some evidence that an aggregation of virus particles plays a small part over this portion of the curve.

The *p*H ranges over which our suspensions remain active is practically the same as that obtained by Stanley (7) where the two sets of experiments are comparable. In Stanley's experiments no reactivation of a completely inactivated suspension was observed on adjusting to a more favourable *p*H value. This is in agreement with our own results in so far as they refer to a completely inactivated suspension. The reactivation demonstrated in an earlier section was obtained with suspensions which were not completely inactivated and was demonstrable only during the first few hours after the preparation of the suspensions. The longer time intervals between Stanley's inoculations would therefore account for the absence of a reactivating effect in his experiments. The absence of such an effect at his first inoculation may be the result of a high initial virus concentration, in which case substantial changes in the concentration of active virus units could occur without being reflected in the lesion counts.

VII. DISCUSSION

The immediate points of interest arising from the data presented have been discussed in the appropriate sections of this paper, and many others which naturally arise must await further data before they can be properly interpreted, but there are some questions of a more general nature which we believe are worth touching upon at this stage.

The first of these concerns the interpretation of past data and the planning of future work. It is obvious that any tests with chemical reagents made in the past by virus workers without regard to the *p*H value of their inocula must be reinterpreted in the light of these results, and that in testing the effect of any particular treatment it will be necessary to arrange matters so that the effect of *p*H value will be eliminated. This applies also to filtration and purification studies. If, for example, the *p*H value at one stage of a purification process is such that inactivation of the virus results then erroneous conclusions may quite easily be drawn. In studies of dilution curves it has been the custom in the past to make up the suspensions in distilled water. The *p*H value and electrolyte content of such inocula will vary with the relative proportions of infective juice and distilled water, and since both of these factors influence the number of lesions produced such curves will need to be redetermined under more strictly controlled conditions. In fact, any quantitative studies with plant viruses must be preceded by a study of the effect of *p*H value and electrolyte content of the inocula on the number of lesions produced, and any effects should be separated as far as possible into those concerned with the virus itself and those concerned with the test plants.

The fact that the *p*H-activity curves of the two viruses studied in this work are so different in nature suggests this relationship as one means of differentiating between viruses, and as an aid to classification. There are many other applications; for example, tomato spotted wilt virus may easily be removed from a mixture with tobacco mosaic virus by adjusting the *p*H value to (say) 4.

In determining the host range of a virus such as that of tomato spotted wilt or its occurrence in various parts of a plant, it will not be sufficient to get negative results by reinoculation of expressed juice. Such negative tests may be due to an inactivation of the virus by an acid juice and will not be conclusive unless *p*H determinations on the expressed juice shows this to be in the range where the virus remains active. Even then the presence in the juice of other substances which may cause inactivation by other means must not be lost sight of.

Although extreme care is required in applying results obtained *in vitro* to the probable behaviour of a virus in the host plant there can be little doubt that when supplemented by other data it will be possible to determine in what parts of a plant a particular virus may or may not survive on account of the *p*H value normal to the part in question. No definite conclusions can at present be drawn as to the nature of the inactivating processes, but on the whole the evidence points to some process other than a simple coagulation, although differences in the degree of dispersion of the virus complex may account for some of the effects observed with tobacco mosaic virus.

Finally, it is tempting to analyse the foregoing results in their bearing on the nature of the viruses themselves. Although much more information on these and other aspects is required before this can be done without undue speculation several points call for comment.

(1) The activity-pH curves resemble similar curves for enzymes more closely than they do curves for living organisms.

(2) The attainment of what appears to be a steady state at pH 9 for tobacco mosaic virus is very interesting. Since the virus does not multiply *in vitro* at pH values much more favourable than this, we may dismiss the possibility that virus is being formed by a multiplication process as fast as it is being inactivated at this pH value. Since the data make unlikely the supposition that the inocula consist of a mixture of strains of differing resistances, this behaviour is strong evidence for the non-organismal nature of this particular virus.

VIII. SUMMARY

1. The interpretation of lesion counts in terms of the relative concentration of active virus units is discussed in its bearing on our results.

2. The effect of pH value on the activity of the virus of tomato spotted wilt has been examined with the following results:

(*a*) Activity-time curves at constant pH value show that in some instances in the absence of oxygen and at 0° C. suspensions of the virus buffered at pH 7 maintain their activity without loss for at least 11 hours. In other instances a fall in activity sets in after about 6 hours and in others even sooner, but in general suspensions remain at constant activity under these conditions for 6 hours or more. Time-activity curves at pH values of 5·2, 6·0, 8·7 and 9·2 are also recorded.

(*b*) An activity-pH curve for the virus (in the absence of free oxygen and at 0° C.) has been constructed. It has been found that the virus is rapidly inactivated at and below a pH value of 5 and above a pH value of about 10. There is no significant difference in activity over the pH range 6–8·9 for a half-hour or 5-hour contact.

3. A study of the effect of pH value on the activity of ordinary tobacco mosaic virus has revealed the following relationships:

(*a*) Activity-time curves at constant pH value show that:

(i) Suspensions of this virus buffered at pH 7 do not change in activity over a period of at least 24 hours at room temperature (*ca*. 20° C.).

(ii) Suspensions at pH about 9 undergo a rapid fall in activity, but eventually reach a steady state and then remain constant. If the suspensions (at pH 9) are adjusted to pH 7 shortly after preparation a marked reactivation takes place, the difference in activity of the adjusted (to pH 7) and unadjusted suspensions getting progressively smaller with time. It is concluded that portion of the virus was completely and irreversibly inactivated, that the amount so inactivated increased with time, that a small portion was reversibly inactivated (i.e. was reactivated when suspensions were adjusted to pH 7), and that the portion capable of reactivation decreased progressively with time.

(iii) Suspensions at pH 2 when inoculated at this pH value cause significantly fewer lesions than the corresponding control inocula, whereas the activity of suspensions held at pH 2 for various times and adjusted to pH 7 before inoculation is not significantly different from that of the controls at pH 7. The differences are discussed and in part at least ascribed to an effect on the host plants.

(*b*) Two activity-pH curves have been constructed for tobacco mosaic virus. One, when the suspensions were adjusted to pH 7 after 12-hour contact at the test pH values and another when the suspensions were inoculated at the test pH value. It is concluded from these tests that the virus undergoes inactivation above pH 8·2 and below pH 2. The extent of this inactivation varies with the pH value and is complete at pH 11 and 0·5. There is no significant difference in the activity of the virus over the pH range 2–8 in the adjusted suspensions and over the pH range 6–8 for the unadjusted suspensions. Differences between the apparent activity of adjusted and unadjusted suspensions at low pH values are recorded and their probable causes discussed. Between pH values of 2 and 5 these differences are relatively small and are probably due in the main to an effect of the acidity of the inoculum on the host plant.

4. The bearing of these results on other problems connected with plant viruses is briefly discussed, and it is pointed out that in the case of tobacco mosaic virus at any rate they are strong evidence for the non-organismal nature of this virus.

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THE CHARACTERS OF SOME COLIFORM BACTERIA ISOLATED FROM GRASS AND GRASS SILAGE

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It is generally recognized that coliform bacteria may be broadly divided into two groups—those occurring naturally on plant surfaces and in the soil, and those whose natural habitat is the intestine of animals. Some species in the two different categories exhibit such well-marked characters (e.g. in relation to formation of acetyl methyl carbinol, growth in citrate medium, etc.) that their original habitat may be established with reasonable certainty. There are, however, a large number of species exhibiting intermediate characters whose identification in this respect is a matter of some difficulty.

It is to be expected that meadow grass will show the presence of coliform bacteria either of a type indigenous to grass or of types picked up from the neighbouring soil and plant surfaces. Moreover, if the grass has been subjected to grazing it is likely to be contaminated with true intestinal types excreted in the faeces of the animals, and the number of these micro-organisms found on examination of the grass will no doubt vary with the extent of the grazing and the period elapsing between grazing and bacteriological examination. Fresh grass used for silage is therefore certain to show appreciable counts of coliform bacteria, and it is a matter of considerable interest to determine what influence this group of organisms may have in contributing to the subsequent fermentation of the silage.

Previous investigations on the subject do not appear to be extensive. Heineman & Hixson (1) found a member of the *colon-aerogenes* group in corn silage in the early stages only and suggested that the first phase in fermentation, of short duration, was caused by this group and resulted in the formation of acid and gas. Hunter (2), using either corn or peas and oats silage, found coliforms varying from 1000 to 100,000,000 per gram in different samples. In one investigation the numbers fluctuated considerably throughout the ripening period of the silage, but in the remainder, after a high count in the early stages, the numbers decreased rapidly. Ruschmann (5, 6) concluded that representatives of the *coli-*

aerogenes group, together with butyric acid bacteria, yeasts and lactic acid bacteria, probably contribute to the formation of acetic acid in silage. It is of interest to note also that Miehe (3) found a coliform species, *Bacillus coli* forma *foenicola*, to be responsible for the initial heating of hay.

Investigations aimed at identifying species commonly found in silage or establishing the extent to which they take part in its fermentation appear to be lacking. In an attempt to remedy this deficiency to some extent, so far as grass silage is concerned, it was decided to determine the numbers of coliform bacteria present in various samples of fresh grass and in silage at different stages during its fermentation and to isolate and determine the characters of the predominant species.

EXPERIMENTAL

Media and temperature of incubation. The usual presumptive test for coliform bacteria is carried out at 37° C. in lactose-bile-salt broth. Preliminary experiments showed that if parallel tests were made on grass extract in the above medium at 30 and 37° C. respectively the count of gas formers at the lower temperature was nearly always much the higher of the two. The reasons for this are discussed later, but it may be noted here that tests for coliforms in grass and silage should certainly be carried out at 30° C. Solid media found suitable for isolation of separate species or for stock cultivation, particularly for capsulated types, were an extract of haricot beans made up with dextrose and agar, and yeast extract dextrose agar.

Numbers and types of coliforms in fresh grass

Six samples of spring grass were obtained from different localities. Samples 1 and 2 were from areas not grazed for 12 months, samples 3 and 4 from areas grazed by sheep until 2 weeks before cutting, and samples 5 and 6 from areas not grazed for several weeks before cutting. Each sample, consisting of about 2 kg., was well mixed and 50 g. ground in a sterile mortar with 450 ml. of saline warmed to 45° C. Further dilutions were prepared in tubes of saline. Quantities of 1 ml. from each dilution were inoculated into two parallel sets of bile-salt broth tubes, one set being incubated at 30° C. and the other at 37° C. The results are shown in Table I.

It is evident that the number of bacteria capable of giving the usual presumptive test at 37° C. is very small in all samples of grass, whether grazed or ungrazed. On the other hand, the same test at 30° C. shows the presence of considerable numbers of gas producers, sometimes as high as 1,000,000 per gram. Three pure cultures of the organisms responsible were isolated from the highest dilutions of the bile-salt broth showing fermentation at 30° C. in the cases of samples 5 and 6.

Table I

Number of coliforms per gram of fresh grass giving the presumptive test at 30 *and* 37° C. *respectively*

Sample		Number at 30° C.	Number at 37° C.
1	Ungrazed	1,000	Under 100
2	Ungrazed	10,000	Under 100
3	Grazed	1,000,000	100
4	Grazed	100,000	Under 100
5		100,000	Under 100
6		1,000,000	Under 100

Additional strains were obtained as follows: Fresh autumn grass was packed in quantities of 25 lb. in large tins, covered with sand and stored at room temperature (about 15° C.). The whole contents of a tin were periodically used for a sample, from which extracts were prepared as above and plated on dextrose-bean-extract agar. The fresh grass gave large, spreading, translucent, mucoid colonies to the extent of about 200,000 per gram. Samples were plated eight times over a period of 15 days, and it was found that there was an increase in the numbers of this type of colony, particularly at the end of the period when there was a count of approximately 80,000,000 per gram. Thirteen cultures of this type were isolated, and these, together with the three referred to above, were studied in detail and found to fall into two groups:

Group 1 (*fourteen cultures*). Short, Gram-negative, motile, non-sporing rods. On media containing dextrose the growth was mucoid and spreading and the cells showed well-marked oval capsules. On nutrient agar the growth changed to a less mucoid one and capsules usually disappeared. Gelatin was very slowly liquefied. Stab cultures incubated at 20° C. showed a barely visible pit of liquefaction after 18 days, and complete liquefaction occurred only after several weeks.

Litmus milk cultures at 30° C. showed acid and gas formation with complete reduction of the litmus after 48 hours, followed by clotting after about 5 days. Slow peptonization then took place which was considerable after about 4 weeks.

The organisms were positive to the methyl-red test but did not produce indol. Tests by O'Meara's method (4) in dextrose-sodium fumarate broth gave positive results for the formation of acetyl methyl carbinol. Growth occurred in Koser's citrate medium. Nitrates were reduced to nitrites.

Acid and gas were formed from dextrose, lactose, sucrose, maltose, mannite, raffinose, salicin and dulcite and in lactose broth containing 0·5 per cent. of bile salt. No change was produced in inulin or adonite.

The temperature relations of these strains were peculiar. They grew well at 20° C. and rapidly and luxuriantly at 30° C., but refused entirely to grow at 37° C. in any of the media tested. They will thus give the presumptive test for coliforms if the test medium is incubated at 30° C., but not if incubated at the usual temperature of 37° C.

Group 2 (*two cultures*). The reactions of these two strains were identical with those in group 1 with the exception that they did not ferment dulcite.

The characters of these organisms (both groups) show them to be intermediate between the typical *Bact. lactis aerogenes* (V.P. positive, methyl-red negative, citrate positive) and the typical *Bacillus coli* (V.P. negative, methyl-red positive, citrate negative). Werkman & Gillan(7) proposed the new generic name *Citrobacter* for certain intermediate species showing citrate and methyl-red positive qualities and reducing nitrates to nitrites. Members of this new genus, however, are described as not forming acetyl methyl carbinol from dextrose except rarely and then only in traces. The strains from grass described here were all positive to O'Meara's test and so cannot be assigned with any certainty to the genus *Citrobacter*. Their slow proteolytic action, general carbohydrate fermentations and formation of acetyl methyl carbinol are similar to the characteristics of *Bacillus cloacae*, but they are differentiated from this organism by their temperature relations and methyl-red positive character. The apparently wide distribution on grass and the well-marked characters of these strains when freshly isolated perhaps justify a new species name; that of *Bacillus* (*Aerobacter*) *aerogenes graminis* is suggested.

Numbers and types of coliforms in silage

Fresh spring grass was packed[1] in twelve small concrete experimental silos, each holding about ½ ton of material. Bacteriological examinations were made at intervals during the period of fermentation, the contents of a whole silo being used on each occasion. Samples were taken from the top and bottom halves and examined separately, each sample consisting of small portions abstracted with sterile tongs at different depths, combined and mixed. Extracts and dilutions were prepared as above and 1 ml. of each inoculated into (*a*) bile salt lactose broth incubated at 30° C., (*b*) the same medium incubated at 37° C., (*c*) xylose broth incubated at 30° C.

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[1] Under the direction of Dr S. J. Watson for some other experiments undertaken jointly with him.

Table II

Numbers of coliform bacteria per gram of grass silage at intervals during ripening

	Numbers producing acid and gas in following broths					
	Top half			Bottom half		
Age of silage days	Bile salt lactose 30° C.	Xylose 30° C.	Bile salt lactose 37° C.	Bile salt lactose 30° C.	Xylose 30° C.	Bile salt lactose 37° C.
0*	100,000	1,000,000	<100	1,000,000	10,000,000	<100
1	100,000	100,000	<100	1,000,000	1,000,000	<100
2	10,000,000	1,000,000	100	100,000	100,000	100
3	1,000,000	10,000,000	<100	1,000,000	1,000,000	100
4	100,000	1,000,000	100	10,000,000	10,000,000	1,000
5	100,000	10,000,000	100	10,000,000	10,000,000	100
8	100,000	1,000,000	10,000	10,000,000	100,000,000	10,000
11	1,000	<10,000	10	100,000	1,000,000	10,000
17	10,000	100,000	100	100,000	1,000,000	10,000
28	100	<10,000	100	100,000	<10,000	100
43	<100	10,000	10	<100	100,000	10
87	10	1,000	100	100	100,000	1,000
117	10	100	10	100	1,000	100

* Fresh grass.

The number of organisms producing acid and gas in these media are shown in Table II. Three conclusions appear to be justified from these figures: (1) The number of coli-forms capable of forming acid and gas at 30° C. is high in the fresh grass, and this number is maintained or slightly increased in the silage until the 8th day, following which there is a definite decrease. (2) Parallel inoculations into bile salt lactose and into xylose[1] broth show that, in twenty-three tests, sixteen (or 70 per cent.) showed a larger number of gas formers in the latter medium, six (or 26 per cent.) showed an equal number in the two media, and only one sample showed a greater number in the bile salt broth. Inoculations from the highest dilutions of xylose broth showing acid and gas into a fresh tube of bile salt broth resulted in every case in acid and gas formation. These data indicate that bile salt has an inhibitory effect on the growth of some coliform bacteria which results in the presumptive test giving a lower number than the number actually present. In the presumptive tests made by Hunter (2) for coliforms in silage no depressing effect was noted in lactose-bile salt as compared with plain lactose broth. His tests were, however, carried out at 37° C. and on a different type of silage. The inhibitory effect is evidently more pronounced in the older samples when the growth curve of the coliforms in the silage is showing a downward trend, and there are therefore an increasing number of moribund cells present. On being inoculated into a fresh nutrient medium without bile salt the latter are apparently resuscitated and ferment sugar in the usual way, but in the presence of bile salt they are too feeble to overcome its inhibitory effect. (3) The number of coliforms giving the presumptive test at 37° C. is very small in fresh grass, while in the silage the number is always small compared with those giving the test at 30° C. The reason for this was found to be that the predominant strain throughout the period of fermentation of the silage was of the *Bacillus aerogenes graminis* type.

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[1] The xylose broth was sterilized by heat which caused a certain amount of decomposition. It was found subsequently that positive results in this medium do not necessarily mean that the test organism ferments xylose.

Pure strains of predominant coliform bacteria were isolated from the highest dilutions of the bile salt broth showing acid and gas at 30 and 37° C. respectively at various ages of the silage. At the lower temperature tests made on seven isolated cultures showed them all to possess the characters of *B. aerogenes graminis* described above.

Eleven strains were isolated from the tubes at 37° C., and their characters are shown in Table III. It may be seen that nine strains are either typical *B. coli communior* or closely allied forms, one strain corresponds with *B. cloacae* and one strain appears to be a non-liquefying variant of the latter organism.

Table III

Characters of predominant coliforms isolated from bile salt broth at 37° C.

No. of cultures	Motility	Lactose	Sucrose	Dulcite	Adonite	Nitrates reduced	Methyl red	Indol	O'Meara's test	Gelatin liquefaction
3	+	+	+	+	−	+	+	+	−	−
2	+	+	+	+	−	+	+	−	−	−
4	+	+	+*	+	−	+	+	−	−	−
1	+	+	+	+	−	+	−	−	+	+
1	+	+	+	+	−	+	−	−	+	−

* Only slight gas evolved.

Discussion

It is evident that the type of coliform bacterium which is apparently widely distributed on grass does not increase in numbers to any great extent in silage and shows a marked decline after 8–10 days. The decrease in numbers is more pronounced in the upper layers than in the lower. These facts are probably explained by the temperature limits for growth of these micro-organisms in relation to the temperature of the silo contents. Readings taken by inserting thermometers when opening each silo showed that the temperature in the top half rose to a maximum of 39° C., attained on the 4th day, while the bottom half reached a temperature of 33° C. on the 17th day. The actual temperatures attained in the silage were no doubt somewhat higher than those recorded by these methods, and it is fair to assume that the decrease in numbers of the *B. aerogenes graminis* type of organism took place as soon as the temperature in the silage approached 37° C. or higher. This evidently occurred more quickly in the upper than in the lower layers. Before that condition occurred it is possible that this group of micro-organisms contributed to the early stages of fermentation, though the increase in numbers is not so large as to suggest that the contribution was a very important one. The part thus played will presumably depend largely on the time taken by the silage initially to reach the temperature inhibitory to their growth.

The number of coliforms capable of growing at 37° C. was never large enough to be responsible for an appreciable fermentation in the silage.

SUMMARY

The species of coliform bacterium predominant on fresh grass was found to be a capsulated type growing well at 30° C. but unable to grow at 37° C. For this reason it will not be detected by the presumptive test in bile salt-lactose broth at 37° C. The name *Bacillus* (*Aerobacter*) *aerogenes graminis* is proposed for this species.

It is possible that coliforms of the above type contribute to a small extent to the initial fermentation of the silage. Those coliforms giving the presumptive test at 37° C. were found in such small numbers in the silage investigated that their influence was concluded to be negligible.

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A COMPARATIVE STUDY OF LACTOBACILLI FROM GRASS SILAGE AND OTHER SOURCES

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The fermentation of grass silage results, amongst the other changes consequent upon plant respiration and bacterial growth, in a lowering of the *p*H from an initial value of approximately 6·0 to a value between 4·0 and 4·5, normally reached after the first few days. The chemical quality and feeding value of the final product, used after a ripening period of 3–6 months, depend upon the fermentation having been controlled in such a way as to preclude excessive loss of proteins and carbohydrates and formation of large quantities of volatile acids. In this control lactobacilli may play an important part. Most species show vigorous growth under conditions of comparatively high hydrogen-ion concentration, and some will flourish at as low a *p*H as 3·5. They result in only slight proteolysis, and the products of carbohydrate metabolism consist mainly and, in many cases, almost entirely of lactic acid, which not only tends to reduce the *p*H still further so as to inhibit many types of spore-forming anaerobes but also has a definite feeding value. The work of Fred *et al.* (4) and of Peterson *et al.* (10) has indicated the influence of this group of bacteria in the fermentation of corn silage and, although they found definite effects in inoculation experiments with *Lactobacillus bulgaricus* and *Bacillus lactis acidi*, they state that in uninoculated corn silage the species present in the largest numbers was *Lactobacillus pentoaceticus*, a type which forms, in addition to lactic acid, appreciable quantities of CO_2, alcohol and volatile acids.

For these reasons, and as a preliminary to inoculation experiments with suitable species, it was decided to study in detail the lactobacilli isolated from several types of grass silage and to compare them with species isolated from other sources. The silages were all made from young grass. The high protein content of this material increases the possibility of deleterious proteolysis and renders it all the more essential that the course of fermentation should be controlled to prevent it.

EXPERIMENTAL

Sources of isolated cultures. Six different grass silages were used, the first five being made from autumn grass subjected to different treatments during ensilage: (1) ordinary grass, (2) whey silage, containing 2·5 per cent. of added whey paste or 1 per cent. lactose, (3) molasses silage, containing 2 per cent. of added molasses, (4) acid molasses silage, to which 4·4 per cent. of 1·67 N hydrochloric acid and 0·7 per cent. of molasses had been added, (5) A.I.V. silage. Each of these was sampled when 3–5 months old. The sixth silage was made from spring grass, and samples were taken for examination at frequent intervals during the course of fermentation.

Other sources used for comparative purposes were Cheddar cheese, a "lactic" soft cheese and Laban-el-Zabady (an Egyptian fermented milk).

Methods of isolation. 50 g. of silage were extracted in a mortar with 450 ml. of saline and successive dilutions of this extract prepared in tubes of saline. Pure cultures were isolated by one of three methods: (1) by inoculating 1-ml. quantities of the dilutions into tubes of yeast-extract-dextrose broth, which were incubated anaerobically at 30° C. for 10–15 days. The highest dilution showing growth was then plated on beerwort agar or on yeast-extract-dextrose-peptone agar, covered in each case by a second layer of the sterile medium; (2) by plating the dilutions of the extracted material directly in double layers of the solid media as above; (3) by preparing shake cultures from the dilutions in either of the above two solid media, buffered to a pH of 4·2 by addition of citric acid and potassium citrate as recommended by Davis (2). Single colonies obtained by each method, after suitable incubation periods, were transferred to broth. Methods (1) and (3) were found to be the best for general use, since method (2) allowed the growth of considerable numbers of other types of bacteria. The shake cultures at pH 4·2 allowed only yeasts and surface moulds to grow in addition to lactobacilli, and since, in the materials tested, the former were present in comparatively small numbers the latter were easily isolated from the higher dilutions in a condition of purity. On the other hand, comparison of this method of isolation with the use of broth cultures under anaerobic conditions showed that the former allowed growth of only a fraction of the total numbers of lactobacilli present owing to the inhibitory effect of the low pH. This effect is illustrated by the figures in Table I, which shows the counts of lactobacilli obtained by the two different methods from the same silage extract in each case at intervals during the ripening period of normal grass silage. From these results it would appear that, as the numbers of lactobacilli in the silage increased, the proportion able to resist acid conditions also increased. That the inhibitory effect of low pH is much greater in the case of very young cells than in older ones was shown by preparing two parallel sets of shake cultures from the same strain (in

Table I

Comparative numbers of lactobacilli (per gram of silage) obtained by two different methods from the same silage extracts

Age of silage (days)	Shake cultures *p*H 4·2	Anaerobic broth cultures *p*H 7·0	Age of silage (days)	Shake cultures *p*H 4·2	Anaerobic broth cultures *p*H 7·0
0	10	10^6	11	210,000	10^8
1	10	10^6	17	520,000	10^9
2	10	10^6	28	9,300,000	10^9
3	300	10^6	43	1,230,000	10^7
4	2600	10^7	87	2,200,000	10^6
5	3500	10^7	117	400,000	10^5
8	3700	10^8			

yeast-dextrose broth) of *Lactobacillus plantarum* at intervals during its incubation at 37° C. One set was prepared at *p*H 4·2 and the other at *p*H 7·0. Table II shows the counts obtained in each case. The effect here is no doubt mainly due to the fact that, as the broth culture aged, the acid formed as a metabolic product killed the less acid-resistant cells, leaving an increasing proportion able to withstand a *p*H of 4·2.

Table II

Duplicate counts (thousands per ml.) of Lactobacillus plantarum *in shake cultures at different pH values*

Age of culture (days)	Count at *p*H 7·0		Count at *p*H 4·2	
	I	II	I	II
1	53,000	54,000	610	250
2	23,000	30,000	1900	2000
3	7,100	6,800	6500	6100
4	4,600	4,000	3100	2900
6	4,300	4,700	3400	3800
8	4,200	3,300	2000	2900
10	3,500	3,000	2200	2400
13	3,500	3,400	1400	2100

A further point of importance is the influence of incubation temperature on the count obtained in acid shake cultures. Table III shows the counts in two parallel sets of shake cultures prepared from the same silage extract, one set being incubated at 30° C. and the other at 37° C. The inhibitory effect of low *p*H is evidently greater at the higher temperature. That the difference in numbers was not due to the presence of strains unable to grow at 37° C. under normal conditions was shown by isolating a number of pure strains from the 30° C. tubes and testing them in neutral broth at 37° C. when satisfactory growth was obtained.

It is clear that acid shake cultures cannot be relied upon to give even an approximate idea of the total numbers of lactobacilli present and, for this reason and in order to be certain of isolating predominant types, the method of anaerobic broth cultures is to be preferred.

Table III

Effect of incubation temperature on the count (thousands per gram of silage) of lactobacilli in shake cultures at pH 4·2

Silage sample	Count at 30° C.	Count at 37° C.
1	2400	770
2	190	27
3	9300	3400
4	104	9·1
5	1230	69
6	5000	230
7	2200	610

Methods of comparison and identification. The absence of biochemical activity exhibited by the lactobacilli, except in the direction of carbohydrate decomposition, which in any case results in a restricted number of metabolic products, renders their identification difficult. The following simple media were first used in order to test the reactions of isolated cultures: litmus milk, litmus milk containing 0·3 per cent. yeast extract, the same two media containing 1 per cent. added dextrose, and various sugar broths containing, in addition to the test sugar, litmus, peptone and 0·3 per cent. yeast extract. Sterilization of most of these was accomplished satisfactorily by steaming but, in the case of a few media, notably xylose and arabinose broths, it was found necessary to sterilize by filtration through a Berkefeld filter to avoid decomposition of the sugar by heat. The reactions in milk media and in dextrose, lactose, sucrose and maltose broths, of strains isolated from the various sources are shown in Table IV, the rapidity of acid formation in milk media being indicated approximately by the number of *plus* signs.

It may be seen that the cultures can be divided into five groups, most of them being contained in the first four. The growth in milk media of all those isolated from silage was stimulated to a varying extent by yeast extract. Strains in groups 1 and 2 showed slow growth in milks without yeast extract, group 2 being differentiated from group 1 by inability to ferment sucrose broth. Group 3 exhibited no growth in milk media without yeast extract and required the addition of both yeast extract and dextrose to give good growth. Strains in group 4 showed no growth in plain litmus milk, were stimulated by the addition of either yeast extract or dextrose, and still more so by the addition of both substances together. Inability to grow in milk in the case of groups 3 and 4 was obviously not due to inability to ferment lactose, though, in fact, strains in these groups usually fermented lactose broth more slowly than dextrose broth. Moreover, in the case of group 4 at least, this inability was not due to the inadequacy of caseinogen as a source of nitrogen, since the growth was stimulated by dextrose. It seems probable that the growth-encouraging properties of both yeast extract and of dextrose are partly due to the influence of these substances in lowering the oxidation-reduction potential.

Table IV

Acid formation by lactobacilli in carbohydrate media

Group	Source	No. of strains	Litmus milk media				Sugar broths			
			Litmus milk	Yeast litmus milk	Dextrose litmus milk	Yeast dextrose litmus milk	Dextrose	Lactose	Sucrose	Maltose
1	Spring silage	52	+	+ + +	+	+ + +	+	+	+	+
	Autumn silage	6								
	Whey silage	1								
	Molasses silage	5								
	Acid molasses silage	5								
	A.I.V.	1								
2	Spring silage	31	+	+ + +	+	+ + +	+	+	–	+
	Molasses silage	5								
	Egyptian fermented milk	1								
3	Spring silage	12	–	– or slight acid	–	+ +	+	+	–	+
	Acid molasses silage	3								
	Cheddar cheese	1								
	Lactic cheese	1								
4	Spring silage	30	–	+	+	+ +	+	+	–	+
	Whey silage	1								
5	Cheddar cheese	2	+ + +	+ + +	+ + +	+ + +	+	+	+	+

Group 5 consists of two strains isolated from Cheddar cheese, able to grow well in milk media, unaffected by yeast extract or dextrose, and able to ferment a wide range of carbohydrates. Davis (3), in a study of the use of similar litmus milk media for the diagnostic culture of lactic acid bacteria, found that *L. plantarum* was stimulated by yeast extract, and that several other plant strains of lactobacilli were stimulated by dextrose. Typical milk bacteria he found to be unaffected by addition of either yeast extract or dextrose.

A number of strains (mainly from spring grass silage) was selected from each of these groups and tested in xylose, arabinose and salicin broths. The pentoses were used particularly because they are known to be formed by degradation of plant carbohydrates, and it has been found by Peterson & Fred (8) that the pentose-fermenting bacteria play a large part in the formation of corn silage. The results are shown in Table V.

It appears that less than 30 per cent. of the silage lactobacilli fermented xylose. The majority fermented either arabinose or salicin or both.

Table V

Fermentation characters in xylose, arabinose and salicin broths

Group	Source	Number of strains	Xylose	Arabinose	Salicin
I	Spring silage	4	+	+	+
	Autumn silage	1			
	Acid molasses silage	1			
	Cheddar cheese	2			
II	Spring silage	4	+	+	–
	Acid molasses silage	1			
III	Spring silage	17	–	+	+
	Molasses silage	1			
	Whey silage	1			
IV	Spring silage	2	–	+	–
	Molasses silage	1			
V	Spring silage	5	–	–	+
VI	Lactic cheese	1	–	–	–
	Egyptian fermented milk	1			
	Total	42	13	35	32

Identification of metabolic products. In order to secure further data for the comparison and identification of cultures from various silages and other sources it was decided to estimate the relative proportions of lactic and volatile acids formed by dissimilation of dextrose, the optical activity of the former and the type of the latter metabolic product. For this purpose representative cultures from the different sources were inoculated into flasks containing 500 ml. of sterile yeast-extract-dextrose-peptone broth and 25 g. of calcium carbonate. These were incubated at 30° C. for upwards of 4 weeks and shaken each day to neutralize developed acidity. The culture was then filtered, the precipitate washed and the volume of filtrate made up to 500 ml. Metabolic products were identified and estimated as follows:

Lactic acid. 50 ml. of filtrate were distilled to half-volume to remove aldehydic substances and the residue was treated with zinc sulphate and NaOH to remove proteins, and copper sulphate and lime to remove carbohydrates. Lactic acid was estimated in an aliquot portion of the supernatant liquid by the method of Friedemann & Graeser (6).

For extraction of the lactic acid 150 ml. of culture filtrate were treated with 20 ml. of strong sulphuric acid and the precipitated calcium sulphate removed by filtration. At first the method used by Orla-Jensen (7) and Van Niel (11)—evaporation of the acidified filtrate on a water bath to a small bulk, admixture of excess anhydrous sodium sulphate and extraction of the solid mass with ether in a Soxhlet apparatus—was adopted, but subsequently it was found more convenient to use a wet extraction method. For this purpose the acidified filtrate was extracted with six to eight successive portions of 100 ml. of ether in a separating funnel, the ether extracts being combined and evaporated on a water bath. The lactic acid yielded by either method was taken up with a small quantity of water and boiled with zinc oxide and charcoal until neutral to litmus. Zinc lactate was then recovered from the filtrate in three or four successive fractions by evaporation, and each fraction was purified three times by recrystallization. To determine whether the zinc salts so obtained were active or inactive the method recommended by Fred *et al.* (5) was used. The material was dried in a desiccator for 4–5 days and the moisture content determined after heating at 105–110° C. for 2½–3 hours. The moisture content of active zinc lactate is 12·85 per cent., that of the inactive 18·16 per cent. Fractions found by this method to be active were submitted to polarimetric tests, the rotation of the lactic acid formed by the culture being taken as opposite to that of the zinc salt.

Volatile acids. 75 ml. of culture filtrate were treated with 5 ml. of sulphuric acid and 20 ml. of water and volatile acids estimated and identified by the method described in a previous communication (Allen & Harrison (1)).

Alcohol. In the case of culture 19 (Table VI) an appreciable quantity of volatile acids and comparatively small amounts of lactic acid were formed. This strain differed further from other cultures tested in producing in the chalk dextrose broth a strong unpleasant cheesy odour reminiscent of sour silage. The distillate obtained from the neutralized culture was found to be free from acetone and almost free from aldehydes but to contain alcohol. A second flask of the same culture was used to concentrate the neutral volatile distillate. Proteins were removed by means of zinc sulphate and NaOH and the filtrate distilled to half-volume three times in succession. The iodoform test was positive at 60° C. and negative at 20° C. The specific gravity of the concentrated distillate corresponded to an alcohol content of 0·3 per cent. in the original culture.

In all the cultures tested, with the exception of No. 19, Table VI, the Duclaux figures showed that the volatile acids consisted almost entirely of acetic acid with only traces of other acids. Where a culture formed active lactic acid, alone or mixed with the inactive type, this was found in every case to be dextro-rotatory.

The results for moisture contents of zinc lactates, percentages of

Table VI

Metabolic products formed from dextrose by selected cultures of lactobacilli. Percentage moisture contents of zinc lactate and relative percentages of lactic and acetic acids

Culture	Source	% moisture content of zinc lactate Fraction			% acid formed from dextrose		
		1	2	3	Lactic	Acetic	Ratio lactic/acetic
1	Spring grass silage (3 days)	18·27	18·24	—	—	—	—
2	,, ,, (8 days)	18·34	18·31	18·24	—	—	—
3	,, ,, (8 days)	18·16	18·01	18·15	—	—	—
4	,, ,, (87 days)	18·21	18·38	—	—	—	—
5	,, ,, (87 days)	18·32	16·29	—	—	—	—
6	,, ,, (87 days)	18·30	18·37	—	—	—	—
7	,, ,, (117 days)	18·17	17·90	12·88	—	—	—
8	Autumn grass silage	18·28	18·24	18·33	2·41	0·166	100/ 6·9
9	Molasses silage	18·41	18·70	18·21	2·61	0·072	100/ 2·8
10	,, ,,	13·11	13·05	13·02	4·57	0·046	100/ 1·0
11	,, ,,	18·02	18·04	—	2·56	0·161	100/ 6·3
12	Acid molasses silage	18·03	—	—	1·26	0·259	100/20·6
13	,, ,,	18·00	18·12	18·16	2·57	0·076	100/ 3·0
14	Whey silage	18·65	17·09	12·38	2·54	0·078	100/ 3·1
15	Cheddar cheese	12·96	—	—	3·71	0·079	100/ 2·1
16	,, ,,	18·12	13·17	12·90	3·38	0·146	100/ 4·3
17	Lactic cheese	18·28	18·07	18·45	3·29	0·070	100/ 2·1
18	Egyptian fermented milk	18·29	18·13	—	1·83	0·255	100/13·9
19	Whey silage	Forms lactic acid (43·3 ml. $N/10$), volatile acids (35·8 ml. $N/10$) and alcohol (0·3 %)					

lactic and acetic acids formed from dextrose, and the ratios of the two latter figures are shown in Table VI for nineteen selected cultures. In Table VII are shown the reactions of these same cultures in milk media and in various broths.

CONCLUSIONS

The results demonstrate the fact that, for adequate comparison and identification of lactobacilli, a considerable amount of information is necessary. Thus complete correlation is rarely possible between results of analysis of metabolic products and reactions in milk media and sugar broths, nor do either of these appear to bear any constant relation to the source of the culture. Two of the three cultures from Cheddar cheese fall into Davis' (3) category of typical milk bacteria; they have identical reactions in litmus milk media and sugar broths, and the one of these which was tested formed only active lactic acid. On the other hand, the third strain from Cheddar cheese (No. 16) only grows satisfactorily in milk after the addition of yeast extract and dextrose and produces from dextrose a mixture of approximately 20 per cent. inactive and 80 per cent. active lactic acid. It is identical in milk and sugar reactions with strain

Table VII

Reactions of selected cultures of lactobacilli in milk media and in various broths

		Litmus milk media				Sugar broth						
Culture	Source	Litmus milk	Yeast litmus milk	Dex-trose litmus milk	Yeast dextrose litmus milk	Dex-trose	Lactose	Sucrose	Maltose	Xylose	Ara-binose	Salicin
1	Spring grass silage	–	+	–	+ +	+	+	–	+	+	+	–
2	,, ,,	–	+	–	+ +	+	+	–	+	–	+	+
3	,, ,,	+	+ + +	+	+ + +	+	+	–	+	–	–	+
4	,, ,,	+	+ + +	+	+ + +	+	+	+	+	–	+	+
5	,, ,,	+	+ + +	+	+ + +	+	+	–	+	+	+	–
6	,, ,,	+	+ + +	+	+ + +	+	+	–	+	+	+	+
7	,, ,,	–	+	+	+ +	+	+	–	+	+	+	+
8	Autumn grass silage	+ +	+ + +	+ +	+ + +	+	+	+	+	+	+	+
9	Molasses silage	–	–	+	+ +	+	+	–	+	–	+	–
10	,, ,,	+ +	+ + +	+ +	+ + +	+	+	–	+	–	+	+
11	,, ,,	+ +	+ + +	+ +	+ + +	+	+	+	+	+	+	+
12	Acid molasses silage	–	–	+	+ +	+	+	–	+	+	+	–
13	,, ,,	+	+ +	+	+ + +	+	+	+	+	+	+	+
14	Whey silage	+	+ + +	+	+ + +	+	+	+	+	+	+	+
15	Cheddar cheese	+ + +	+ + +	+ + +	+ + +	+	+	+	+	+	+	+
16	,, ,,	–	+	–	+ +	+	+	–	+	–	–	–
17	Lactic cheese	–	+	–	+ +	+	+	–	+	–	–	–
18	Egyptian fermented milk	+ +	+ + +	+ +	+ + +	+	+	–	+	–	–	–
19	Whey silage	–	+ + +	+	+ + +	+	+	+	+	–	+	+

17 from "lactic" cheese which, however, forms only inactive acid. Cultures 8 and 11 correspond in milk and in sugar reactions, both form only inactive lactic acid, and the proportions of lactic and acetic acids are very similar; they appear to be identical strains. Cultures 13 and 14 correspond in all particulars except that the latter forms a small proportion of active lactic acid. The strain from Egyptian fermented milk is stimulated in milk to only a slight extent by yeast extract or dextrose and therefore may be regarded as a typical milk strain. Its sugar reactions are identical with those of Nos. 16 and 17 from cheese, but it differs from these strains in other particulars.

Of the fifteen strains from silage which were tested ten form only inactive lactic acid, three form mainly inactive and a small proportion of active acid (dextro), while one forms only *d*-lactic acid. These do not develop appreciable quantities of gas in carbohydrate fermentation, the majority ferment salicin and find yeast extract a particularly suitable source of nitrogen. They therefore correspond with Orla-Jensen's (7) genus *Streptobacterium*, and some of the strains agree in their general reactions with his description of *S. plantarum*. Culture 10, since it forms only *d*-lactic acid, grows well in milk and is not specially stimulated by yeast extract, appears to correspond with this author's *S. casei*. However, in view of his statement that the genus *Streptobacterium* contains many species which are difficult to distinguish from one another owing to the gradual transition between them, it is perhaps unwise to allot specific names.

The strains obtained from grass and silage appear to find their analogues in some of those occurring in dairy products, which indicates the probable habitat of some of the lactobacilli playing a part in the ripening of these.

The remaining culture (No. 19), obtained from silage, forms besides lactic acid appreciable amounts of other metabolic products—volatile acids and alcohol—does not grow in milk and ferments pentoses. It corresponds in these particulars with Orla-Jensen's genus *Betabacterium* and is similar to *Lactobacillus pentoaceticus* described by Peterson & Fred (9). The Duclaux figures for this strain corresponded with those for a mixture of three parts acetic acid and one part butyric acid. This proportion seemed to be remarkably constant, since the figures from two separate flask cultures of different ages were identical. The rare occurrence of this type in grass silage points to a considerable difference between the lactic acid flora of this and of corn silage where *L. pentoaceticus* was found by Fred *et al.* (4) to assume a prominent place 12 days after ensiling and to form 50 per cent. or more of the total flora after 8 or 10 weeks.

In general it may be said that the great majority of lactobacilli in the different grass silages investigated were of the type which form from carbohydrates mainly lactic acid (usually inactive) with only a small quantity of acetic acid. They all appear to ferment lactose and maltose, the majority form acid in salicin or arabinose but they exhibit wide differences in their ability to ferment other carbohydrates. About 30 per cent. of strains form acid in xylose and rather less than 50 per cent. in sucrose. Their sensitivity to the nitrogen source shows considerable variation, but all strains are stimulated to a greater or less extent by yeast extract.

Summary

Samples of 152 strains of lactobacilli isolated from six different types of grass silage, which had been subjected to various treatments at the time of ensiling, and five strains isolated from other sources for comparative purposes, were tested by their reactions in litmus milk, yeast extract litmus milk, dextrose litmus milk, yeast extract dextrose litmus milk and in various carbohydrate broths. The metabolic products formed from dextrose by nineteen selected strains were identified and estimated.

The evidence obtained in this way pointed to the conclusion that the majority of lactobacilli in grass silage are strains of *Streptobacterium plantarum*, characterized by comparative inactivity in litmus milk, but markedly stimulated by addition of yeast extract. They produce from dextrose mainly lactic acid (usually the inactive form) with a small quantity of acetic acid. A strain was encountered which also produced alcohol and butyric acid, but the numbers of this type in silage were negligible. Considerable differences were found in the ability of the various strains to ferment carbohydrates.

Of the five strains isolated from dairy products three were of the *Streptobacterium plantarum* type and two were of a kind which rapidly clotted litmus milk and exhibited reactions corresponding to the true milk strains.

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STUDIES ON EUROPEAN FOUL BROOD OF BEES

II. THE PRODUCTION OF THE DISEASE EXPERIMENTALLY

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(With Plates XXII–XXIV)

The etiology of so-called European foul brood of bees has long remained in doubt in spite of considerable investigation. Since numerous summaries of the pertinent literature are available (3, 4, 5, 10, 15, 16) an exhaustive résumé of the historical aspects of the problem is unnecessary, and in this paper, therefore, the results of a number of experiments dealing with the production of the disease are discussed in detail with reference to their value in establishing its cause.

Experimental

Media for cultivation of the bacteria

Hartley's tryptic digest (beef) broth was employed, and agar (1·5 per cent.) and gelatin (12 per cent.) media were prepared from this. Sturtevant's egg agar (egg-yeast-carrot-peptone) was used (14). "Brood-filtrate agar" was prepared by adding 1 ml. of a 1 : 10 solution of brood filtrate (21) to each 5 ml. of beef-digest agar. Nitrate broth was made by adding 0·1 per cent. pure potassium nitrate to the beef-digest broth. The following medium was employed to study hydrogen sulphide formation: Lemco beef extract 1 per cent., peptone (B.D.H.) 2 per cent., lead acetate 0·02 per cent., cystine 0·02 per cent. and agar 1·5 per cent. The cystine was sterilized separately in water and was added as a suspension to the agar medium immediately prior to sloping. All the above media were adjusted to *p*H 7·0 and were sterilized by autoclaving. Fresh skim milk was employed and was sterilized (with or without litmus indicator) by intermittent steaming. A basic medium consisting of Witte peptone 2 per cent., sodium chloride 0·5 per cent., with 4 ml. of an 0·4 per cent. solution of brom cresol purple per litre was employed to detect the formation of acid from various carbon compounds by the bacteria studied. With the exception of raffinose and fructose, which were sterilized by Seitz filtration, all the fermentable compounds investigated were sterilized separately by autoclaving in 4 per cent. aqueous solution, and were added to the basic medium to make a final concentration of 1 per cent. Indole and nitrite were tested for as previously described (16).

Source of infective material and bacterial cultures

All the naturally infected material employed in this investigation was obtained from a colony of hybrid bees in which "European foul brood" had been initiated by the insertion of an infected brood comb in the autumn of 1934. The disease appeared in the spring in several of the larvae which were being reared in the original infected comb, and rapid spread was facilitated by removing a large proportion of the sealed brood combs together with the adhering bees from the colony early in the brood-rearing season, and replacing these with drawn comb or foundation, thus causing extensive weakening of the colony. *Bacillus alvei*, *Streptococcus apis* and a small rod-shaped non-spore-forming bacterium (*Bacterium eurydice*? (20)) were constantly encountered in the infected larvae examined. Larvae were normally attacked when they were about 4 days old, but dark brown, slimy, evil-smelling larvae full of *B. alvei* spores and vegetative cells were fairly frequently encountered in capped cells. In most respects this case of disease conformed with the "European foul brood" described by White as typical (20).

Pure cultures of *B. alvei* were isolated from naturally or experimentally infected larvae by grinding them up in sterile water and "plating" the resulting suspension on brood-filtrate agar after making suitable dilutions. The plates were "dried" at 35° C. for a short time after pouring in order to obviate the "spreading" of surface colonies. Stock spore-containing cultures of the various isolated strains of *B. alvei* were prepared by inoculating egg-agar slopes directly from the isolated colonies which developed on the plates and incubating them for 5–7 days at 35° C. The resulting cultures were sealed with paraffin wax and were stored at room temperature until they could be identified.

Pure cultures of *S. apis* were obtained in much the same manner as those of *B. alvei*, except that recently infected larvae were found to be most suitable for the isolation of this species, and drying of the plates was unnecessary. Stock cultures of the isolated strains were prepared from primary colonies by inoculating brood-filtrate agar slopes, incubating them for 12–16 hours at 35° C., and sealing the resulting culture. Culture 1 (*B. alvei*) and culture 2 (*S. apis*) have been employed in all the infection experiments, and normally all cultures have been incubated at 35° C.

Identification of isolated cultures of Bacillus alvei *and* Streptococcus apis

Streptococcus apis *strains.*

Although Borchert (5) has described *S. apis* in some detail it has been thought advisable to discuss the characteristics of this organism here in view of the fact that certain important differences have been noted among the strains examined. All the media employed in the identification of the various strains were inoculated from 12–24-hour-old brood-filtrate agar cultures which had been inoculated directly from the stock cultures. The number of each culture refers to a given experiment (*vide infra*).

Morphology. On agar media, in broth and in milk, the predominating form is that of a lanceolate-shaped *Diplococcus* (Pl. XXII, figs. 1–4); very occasionally short chains are formed (Pl. XXII, fig. 2). The size of the cells is extremely variable under certain conditions (Pl. XXII, fig. 5). The organism is non-motile, and so far no capsules have been noticed on any of the media. The organism is Gram positive when very young cultures are examined, but the cells rapidly lose their power to retain this stain, and in most cultures an abundance of Gram-negative cells can be seen. A comparison of the cells of this organism with those it forms in larvae sick or dead of "European foul brood" can be obtained from a study of Pl. XXIII, figs. 7, 8, 11 and 12, and Pl. XXIV, figs. 15 and 16.

Cultural and biochemical characteristics. On beef-digest agar the growth is very rapid: it is uniform with regular margin, opaque and rather white, shiny, slightly raised, soft, moist and non-adherent. The addition of brood filtrate accelerates growth and makes it more abundant. Surface colonies on beef-digest agar are circular with regular margin, slightly raised and about 1–2 mm. in diameter. Subsurface colonies are lanceolate shaped. In beef-digest broth growth is followed by uniform clouding of the medium; eventually a heavy precipitate settles, the medium becoming fairly clear. Considerable variation has been found with respect to growth and reaction toward milk and gelatin media. Thus cultures 1, 6, 9 and 11 when grown in litmus milk rapidly reduce the indicator and coagulate the casein, almost completely peptonizing it within a week. After 1 week only a small amount of red undigested casein and a clear almost yellow whey remains. The milk is quite acid. In beef-digest gelatin stab cultures these strains grow quite well at 22° C., and within 48 hours there is a marked saccate liquefaction of the medium. At 35° C. there is rapid and complete liquefaction of gelatin.

On the other hand, cultures 3, 4, 5, 7, 8 and 10 neither hydrolyse casein nor gelatin. Thus growth of these strains in litmus milk is followed by a mere transient reduction of the indicator which soon becomes reoxidized, leaving a faintly acid uncoagulated medium. In gelatin stab cultures at 22° C. the growth is uniform and filiform and no liquefaction occurs. Similarly at 35° C. no liquefaction of the gelatin results even after 3 weeks. None of the strains studied reduce nitrate to nitrite, and none form hydrogen sulphide or indole in old milk cultures or in broth. All strains investigated exhibit the same fermentative power. Thus acid is formed from dextrin, sucrose, lactose, maltose, glucose, fructose, galactose, mannose, mannitol, glycerol and salicin by all the cultures, and none of them produce acid from starch, inulin, raffinose, arabinose, xylose, inositol, adonitol and erythritol under the experimental conditions. Borchert (5) found that the strains of *S. apis* investigated by him produced acid in small amounts from raffinose, but it must be remembered that fermentation reactions of a given species are liable to significant variations depending on the nitrogen substrate employed in its cultivation (11, 12). It is of interest that the species hitherto known as *S. apis* is apparently divisible into subspecies depending on the power of hydrolysing casein and gelatin. In all other respects the various strains studied appear to be identical.

Bacillus alvei *strains.*

All the strains of *B. alvei* isolated (cultures 2, 12, 13, 14, 15, 16, 17 and 18) exhibited the same general cultural, morphological and biochemical characteristics as has been described for certain other cultures (16). The fermentation reactions of all the cultures isolated was found to be identical with those given by cultures 3 and 4 described previously (16), with the single exception that raffinose was not fermented.

Preparation of material for infection experiments

Suspensions of the spores of *B. alvei* (strain 2) and vegetative cells of *S. apis* (culture 1) were prepared as follows. Suspensions of these organisms were prepared in sterile water directly from the original stock cultures in order to avoid too many transfers away from the natural host and the consequent danger of loss of virulence. From these egg agar, in the case of *B. alvei*, and brood-filtrate agar, in the case of *S. apis*, were inoculated employing large amounts of these media in Petri dishes or culture bottles. The resulting cultures were incubated 5–7 days in the case of *B. alvei*, and 12–16 hours in the case of *S. apis*. The spores or vegetative cells were then washed carefully from the medium employing sterile water, centrifuged in order to concentrate them, and finally suspended in water to give a relatively dense suspension. The approximate number of spores or vegetative cells present in such suspensions was estimated by making dilutions and counting the number of bacteria by means of a Thoma haemocytometer slide.

Filtrates of infected brood were prepared by grinding up larvae sick or dead of "European foul brood" in water, adjusting the *p*H of the resulting suspension to 7·2 or 7·4, allowing the mass to stand approximately half an hour at 35° C., centrifuging at a low speed to remove the larger particles of cell debris, and finally filtering the supernatant liquid through Pasteur-Chamberland L 2 or L 3 filters, employing suction. Filtrates obtained in this manner were normally opalescent, and in each instance their sterility was verified by plating 2 ml., employing brood-filtrate agar as substrate and incubating the plates 2 days at 35° C. and 5–7 days at room temperature.

Preparation of experimental nuclei employed in infection experiments

It is naturally impossible to obtain any absolutely standard nucleus or colony of bees for experimental work, since there are so many uncontrollable variables, such as the ratio of "nurse" and "house cleaning" bees to brood at different stages in the brood-rearing season, the relative amount of sealed and unsealed brood present, the effect of honey flow, etc. In addition the race of bee undoubtedly influences the susceptibility of a given colony of bees to "European foul brood", Italian races being particularly resistant in this respect (13). In all the experiments recorded in this paper hybrid bees have been used owing to force of circumstances, and it seems highly probable that the resistance toward this disease displayed by certain nuclei depended to some extent at least on the amount of Italian strain in the bees. Since no standard of strength of an experimental nucleus is available it has not been possible to give an exact statement regarding the strength of a given nucleus in bees, and only in cases where the nucleus was obviously strong or weak in bees has a note to this effect been made. All experiments recorded in this paper have been carried out in single-walled nucleus hives capable of taking five British Standard brood frames. The entrance consisted of a 2 by $\frac{1}{2}$ in. slot cut in the front of the hive, and the frames were covered by a piece of "Ten Test" board which had a $1\frac{1}{2}$ in. diameter hole bored in the centre covered with $\frac{1}{8}$ in. wire gauze for a "feed hole". Each hive was equipped with a deep roof capable of covering the feeder used. Feeding was carried out using 1 or $\frac{1}{2}$ lb. Ministry of Agriculture "squat" honey jars with five $\frac{1}{16}$ in. diameter holes bored in the lids. These were filled with the syrup employed and were inverted over the feed hole. After 15 August all the nuclei were fed with 50 per cent. sucrose syrup in order to stimulate brood rearing.

The nuclei were made up by taking three or four brood combs containing chiefly sealed brood and stores (honey and pollen) together with the adhering bees from healthy stocks, and placing these in nucleus hives. The bees of each nucleus were then permitted to raise their own queen. Owing to unfavourable weather conditions none of the experimental nuclei could be prepared before 23 May, and as a result of continued unfavourable conditions the mating of the queens was delayed and hardly any experiment was commenced prior to the last week in June. However, this resulted in considerable weakening of some of the nuclei with respect to the number of bees present, and consequently they were probably more susceptible to "European foul brood" (*vide infra*). As the season progressed the nuclei were given additional frames containing brood comb or foundation in order that there might be room for expansion of the brood nest.

Method of inoculation of experimental nuclei

Two general methods of infecting nuclei have been attempted; namely, indirect inoculation by feeding the bees the infective material, and direct inoculation by feeding young larvae directly. In the indirect method the infectious material was fed, usually mixed with 30 per cent. sterile sucrose syrup containing 10 parts of honey for every 100 parts of syrup, to the bees of a healthy nucleus by means of a feeder. In the direct method of inoculation the following technique was employed.

Brood combs containing eggs and coiled larvae up to the age of approximately 4 days were obtained from healthy colonies of bees. Experience proved that the best way to obtain combs with an abundance of young larvae was to place a frame containing brood foundation in a vigorous healthy stock and permit the bees to draw out cells and to rear the larvae in them. In this manner brood combs containing an abundance of coiled larvae of a relatively uniform age were readily obtained at any time during the active part of the brood-rearing season. For most experiments, especially those with "starved larvae", larvae about 3–4 days old were inoculated, but occasionally infective material was introduced to the base of cells containing eggs, or into the brood-food mass surrounding very young larvae. The actual inoculation was made by feeding individual larvae either 0·0025 or 0·005 ml. of a suspension of bacteria or infective material by means of an Agla micrometer syringe, the minute drop of liquid being placed near the mouth-parts of the larva with the needle. In certain of the experiments the brood combs containing the inoculated larvae were placed in a healthy nucleus immediately subsequent to inoculation, while in others the larvae were starved in the comb, in an incubator in which the humidity of the air was retained at a fairly high level, for 4 days at 35° C. This starvation technique has been found particularly useful, for the resistance of the larvae is apparently weakened under such conditions, and multiplication of the bacteria in the host is thereby greatly facilitated. In one experiment it is shown that normal coiled larvae are practically sterile bacteriologically. Moreover, it has been observed that, although a very large number of artificially inoculated larvae succumb to infection after starvation, a certain percentage apparently destroy the bacteria, and, at least as far as microscopical examinations are concerned, appear to be sterile bacteriologically after the 4 days. As subsequent experiments will show, inoculated unstarved larvae which are attended to by the nurse bees have a relatively high resistance toward the bacteria. It would seem that larvae possess some potent bactericidal mechanism: perhaps this is merely a function of their digestive system.

Method of examination of inoculated nuclei and of recording experiments

Inspections of the brood of inoculated nuclei were made fairly frequently, care being taken to avoid any undue disturbance of the bees. Where necessary the bees were smoked or shaken from the combs in order to facilitate inspection of the larvae. All larvae which appeared to be diseased, unless there were a very large number, were removed from the cells with sterile forceps, placed in Petri dishes, and taken to the laboratory for examination. In cases in which a very large number of affected larvae were present only a small fairly representative number were examined. Though ordinary microscopical examination of nigrosine or Gram-stained smears from the tissues of affected larvae can usually be relied upon as a satisfactory confirmation that "European foul brood" is present in a nucleus, diagnosis was made more certain by the isolation and identification of one pure culture of *B. alvei* and *S. apis*. In the record of the experiments the term "*S. apis* cells" indicates that lanceolate-shaped cocci of the type shown in Pl. XXIII, figs. 7, 8, 11 and 12, were present in the tissues of affected larvae. Similarly the term "*B. alvei* cells and spores" indicates that vegetative cells or spores of this organism as shown in Pl. XXIII, figs. 11 and 12, and Pl. XXIV, figs. 14 and 15, were present in affected larval tissues. The "small rod-shaped bacterium" is probably synonymous with White's *Bacterium eurydice* (20). In each experiment the strength of the nucleus employed in stores (honey and pollen) and brood (eggs, young larvae and sealed brood) is recorded; thus the record "stores; eggs and young larvae; young larvae; sealed brood", indicates a nucleus with four brood combs containing the brood or stores as indicated in the combs. The terms "strong" and "weak" with respect to the relative number of bees present in a given nucleus have only been employed where it is very evident that such a condition exists. The date of inoculation has been recorded for each experiment in view of the variation in the ratio of "nurse" and "house cleaning" bees to young larvae at different periods in the brood-rearing season, and the probable influence of this ratio on the susceptibility of a given nucleus to "European foul brood". The data obtained in each experiment has been arranged according to the following plan: (1) Nucleus, (2) Inoculum, (3) Date of inoculation, (4) Results.

RECORD OF EXPERIMENTS

EXP. 1.

Ten larvae approximately 3–4 days old (average weight calculated from twenty larvae was 42 mg.) were carefully removed from their cells employing sterile forceps and were ground up in 10 ml. of sterile water observing aseptic precautions. 0·1 and 1 ml. portions of the resulting suspensions were plated, using 10 ml. of beef-digest agar in each instance. After 48 hours at 35° C. only two colonies developed on the plate which had received 1 ml. of suspension. This result would seem to indicate that normal healthy larvae are almost bacteriologically sterile, but further investigation is required in confirmation of the experiment.

I. *Inoculation of experimental nuclei by placing in them brood combs containing naturally or artificially infected larvae, or other form of infective material*

EXP. 2.

Nucleus. Stores; eggs and young larvae; infected comb; sealed brood; sealed brood and stores. Weak in bees.

Inoculum. Brood comb containing approximately eighty-four larvae sick or dead of "European foul brood".

Date of inoculation. 9 July.

Results. 3 *days*: Most of the infected larvae had been removed from the introduced comb and eggs had been laid in the empty cells. 7 *days*: No diseased larvae in the introduced comb; three sick or dead larvae in each of the two adjacent combs. Two of these were full of *S. apis* cells and the remaining four showed *S. apis* and *B. alvei* vegetative cells. 10 *days*: Twenty affected larvae in the introduced comb, fifteen in the remaining combs. Six of these on microscopic examination showed *S. apis* and *B. alvei* cells and small rod-shaped bacteria. 13 *days*: Fifty affected larvae in the introduced comb. Ten of these were examined and showed *S. apis*, small rod-shaped bacteria, and in the case of old slimy decaying larvae sporulating cells of *B. alvei* in apparently pure culture. 21 *days*: Destroyed. (Queen retained with twelve workers for Exp. 31.) Fifty-seven sick or dead larvae were counted in one brood adjacent to the introduced comb. Ten representative affected larvae were examined microscopically: three recently affected larvae showed *S. apis* cells and a varying proportion of small rod-shaped bacteria; seven slimy, evil-smelling dead larvae, some from sealed cells, showed *B. alvei* vegetative cells and spores in apparently pure culture. *S. apis* (culture 8) and *B. alvei* (culture 12) were isolated in pure culture from affected larvae.

In this experiment very rapid spread of the disease was effected by inserting a comb containing a relatively large number of affected larvae in a healthy nucleus which was rather weak in bees.

Exp. 3.

Nucleus. Stores; eggs and young larvae; inoculated comb of eggs and young larvae; young larvae and sealed brood; eggs and stores.

Inoculum. Ten larvae sick or dead of "European foul brood" were ground up finely in 5 ml. of sterile brood filtrate. Fifty eggs and young larvae up to the age of about 4 days were fed 0·005 ml. of this suspension, and the comb containing them was placed immediately in the nucleus.

Date of inoculation. 19 June.

Results. 2 *days*: No affected larvae seen, though a few larvae had been removed from their cells. Subsequent careful examinations of the brood 4, 8, 14, 15, 17, 19, 22, 29 and 31 days after inoculation revealed no infection.

The failure to initiate infection with naturally infected material makes this experiment rather interesting. The bees and the queen of the nucleus used were yellow and probably possessed a relatively large proportion of Italian blood, and the failure may well have been due to the strong cleaning powers of the bees.

Exp. 4.

Nucleus. Stores; young larvae (inoculated comb); young larvae and stores.

Inoculum. Fifty larvae sick or dead of "European foul brood" were finely ground in 10 ml. of sterile water. 2000 2–3-day-old larvae on one side of a brood comb were each fed 0·005 ml. of this suspension, and the comb containing them was placed in the nucleus immediately.

Date of inoculation. 2 July.

Results. 2 *days*: No sign of diseased larvae. A large number of the inoculated larvae had been removed by the bees and eggs laid in the empty cells. 3 *days*: No sign of diseased larvae. Approximately half the inoculated larvae had been removed by the bees, the remainder were being reared in the normal manner and many were sealed. No sick or dead larvae were noticed during inspection of the brood 5, 8, 10, 12 and 17 days after inoculation. 28 *days*: Four sick larvae. All of them had an abundance of *S. apis* cells in their tissues, and two of them *B. alvei* cells as well. 41 *days*: Three sick larvae full of *S. apis* cells were recovered from one comb. *S. apis* (culture 9) was isolated in pure culture from one of these. 46 *days*: Seven affected larvae were recovered. All of these showed *S. apis* cells and small rod-shaped bacteria, and several of them *B. alvei* cells as well. *B. alvei* (culture 16) was isolated from the remains of one dead larva. 55 *days*: Four sick larvae full of *S. apis* cells, small rod-shaped bacteria and *B. alvei* cells. 60 *and* 65 *days*: The bees were breeding very slowly and no infected larvae were seen.

In this experiment therefore the disease was merely transient and never assumed serious proportions.

Exp. 5.

Nucleus. Stores; young larvae (inoculated comb); young larvae and sealed brood.

Inoculum. 500 eggs and young larvae 1–4 days old were each given 0·005 ml. of a suspension of *S. apis* cells, each receiving approximately $10{\cdot}4 \times 10^7$ cells of this organism. The comb was then placed in the nucleus immediately after inoculation.

Results. 2 *days*: Very few of the larvae had been removed by the bees, and there was no sign of infection. 3 *days*: A large number of the inoculated larvae had been sealed over by the bees. Subsequent examinations of the brood 6, 8, 10, 13, 18, 20 and 21 days after inoculation revealed no infected larvae.

Exp. 6.

Nucleus. Stores; eggs and sealed brood; eggs and young larvae (inoculated comb).

Inoculum. 350 eggs and young larvae 1–4 days old were each given 0·0025 ml. of a very dense suspension of *S. apis* cells, each larva receiving approximately 12×10^8 organisms. The comb containing the inoculated larvae was then placed immediately in the experimental nucleus.

Date of inoculation. 5 July.

Results. 2 *days*: A few of the eggs and young larvae had been removed by the bees, no infected larvae were seen. Subsequent inspection of the brood at intervals of 4, 5, 8, 10, 13, 17, 20 and 24 days after inoculation revealed no sick or dead larvae.

Exps. 5 and 6 indicate that the individual larvae have a high resistance toward *S. apis* vegetative cells in very large numbers.

Exp. 7.

Nucleus. Sealed brood; eggs and young larvae (inoculated comb); sealed brood; stores.

Inoculum. 300 eggs and young larvae from 1–4 days old were each given 0·005 ml. of a suspension of *B. alvei* spores, each larva receiving approximately $5{\cdot}4 \times 10^7$ spores. The comb was immediately suspended in the nucleus.

Date of inoculation. 13 June.

Results. 4 *days*: An almost perfect patch of sealed brood present at the site of inoculation, indicating that very few larvae, if any, had been removed by the bees. Careful inspection of the brood at intervals of 9, 14, 21, 27, 32, 37, 42, 48, 55, 58 and 73 days after inoculation revealed no infected larvae.

Exp. 8.

Nucleus. Young larvae and eggs (inoculated comb); sealed brood; young larvae and eggs. Stores present in the brood combs.

Inoculum. 450 young larvae up to 4 days of age and some eggs were given 0·0025 ml. of a very dense suspension of *B. alvei* spores, each receiving approximately $2{\cdot}7 \times 10^8$ spores. The comb containing these was immediately inserted in the nucleus.

Date of inoculation. 5 July.

Results. 2 *days*: A few larvae had been removed from the cells in the area inoculated but there was no sign of any infected larvae. No infected larvae were seen at subsequent examinations of the brood 4, 5, 10, 14, 20, 25, 28, 33 and 34 days after inoculation.

These two experiments indicate that young larvae when reared normally by nurse bees have a high resistance toward *B. alvei* spores in very large numbers.

Exp. 9.

Nucleus. Stores; eggs and young larvae; eggs and young larvae (inoculated comb); sealed brood.

Inoculum. Twenty "European foul brood" larvae in all stages of disease were triturated with 20 ml. of sterile water, and from the resulting mass a Pasteur-Chamberland L 3 filtrate was obtained as previously described. A portion of this filtrate was mixed with an equal amount of a suspension containing $3\cdot5\times10^{10}$ *B. alvei* spores per ml. Each of 100 larvae 2–4 days old on one side of a brood comb received 0·005 ml. of this suspension, each larva therefore receiving approximately 0·0025 ml. of the filtrate and $8\cdot7\times10^{7}$ spores of *B. alvei*. The comb containing the inoculated larvae was placed in the nucleus immediately subsequent to inoculation.

Date of inoculation. 21 June.

Results. *2 days*: A few of the larvae in the inoculated area had been removed by the bees. Subsequent inspection of the brood 6, 11, 14, 19, 24, 28, 32, 35 and 41 days after inoculation revealed no infected larvae.

The experiment suggests that a filterable virus is not implicated in this disease.

Exp. 10.

Nucleus. Stores; sealed brood; eggs and young larvae; young larvae (inoculated comb); stores. Weak in bees.

Inoculum. Each of 800, 2–3-day-old larvae were fed 0·0025 ml. of a suspension of *S. apis* cells, individual larvae receiving approximately $5\cdot2\times10^{7}$ organisms. The comb containing them was then incubated in an incubator, in which the humidity of the air was maintained at a high level, for 4 days at 35° C. At the conclusion of this time a large proportion of the larvae had become yellow to brown in colour and pappy in consistency and were full of *S. apis* cells. (The appearance of *S. apis* cells in starved larvae is shown in Pl. XXII, fig. 6 and Pl. XXIII, figs. 9 and 10.) However, some of the larvae appeared to resist the bacteria, and although they appeared to be dead they were apparently sterile as far as could be observed from simple microscopical observations. The comb containing the infected larvae was then inserted in the nucleus.

Date of inoculation. 2 July.

Results. *2 days*: All the infected larvae had been removed by the bees, and eggs laid in the empty cells. *5–8 days*: No sign of any infected larvae. *10 days*: Several of the larvae which the bees had reared in the introduced (inoculated) comb appeared to be sick, and two of these on examination proved to be full of *S. apis* cells. *11 days*: One sick larva in the inoculated comb; this one full of *S. apis* cells. A very irregular appearance of the brood in this comb indicated that the bees had removed a very large number of larvae. *16 and 20 days*: No affected larvae seen. *25 days*: Thirty-three sick or dead larvae, mostly about 4 days old, were counted in the brood combs. Ten of these were examined microscopically. All of them contained masses of *S. apis* cells, together with a variable number of small rod-shaped bacteria, and in a few cases *B. alvei*-like organisms. *S. apis* (culture 4) was isolated from one of the recently infected larvae. 29 *days*: Disease very marked and the nucleus was destroyed, the queen and twelve worker bees being used in Exp. 31. A very large number of infected larvae were counted in the brood combs. Thus there were approximately fifty-seven affected larvae in one of the combs adjacent to the one which was originally inoculated. Ten of these were examined. All showed *S. apis* cells in large numbers and a variable proportion of small rod-shaped bacteria, and some *B. alvei* cells. Two slimy evil-smelling larvae showed *B. alvei* cells and spores in apparently pure culture. *S. apis* (culture 6) and *B. alvei* (culture 14) were isolated in pure culture from remains of dead larvae.

EXP. 11.

Nucleus. Stores and sealed brood; eggs and young larvae; young larvae (inoculated comb); eggs, young larvae and stores; sealed brood and stores.

Inoculum. 425 and 530, 3–4-day-old larvae on either side of a comb were each fed 0·005 ml. of a suspension of *S. apis* cells, individual larvae receiving approximately $1{\cdot}6 \times 10^8$ bacteria. The comb containing them was then incubated as in Exp. 10. At the conclusion of this time many of the larvae had been attacked by *S. apis* as in the previous experiment, and the comb was introduced into the nucleus.

Date of inoculation. 2 August.

Results. 4 *days*: Most of the infected larvae had been removed from the introduced comb and eggs laid in the empty cells. 6 *days*: No sign of infected larvae. 11 *days*: Several of the larvae which had been reared in the infected comb by the bees had been removed giving the brood an uneven appearance. One of seven larvae examined was full of *S. apis* cells, the remainder being unaffected. No infected larvae were seen on examinations of the brood 20, 27 and 30 days after inoculation.

The results of the above two experiments show that "European foul brood" can be initiated by placing a large number of larvae artificially infected with *S. apis* in a healthy nucleus, providing this is done early in the brood-rearing season and the nucleus is weak in bees. When the inoculation is carried out late in the brood-rearing season the bees are apparently able to ward off the disease. In the first experiment a mixed bacterial infection of the brood similar to "European foul brood" resulted when *S. apis* was employed alone in the infection of the starved larvae. However, as will be seen from Exp. 12, the relative proportion of *S. apis* cells in affected larvae was greater when *S. apis* was used to infect starved larvae than when *B. alvei* was used.

EXP. 12.

Nucleus. Stores; eggs and young larvae; young larvae (inoculated comb); eggs and stores. Weak in bees.

Inoculum. 180 and 395, 3–4-day-old larvae on either side of a comb were fed 0·005 ml. of a suspension of spores of *B. alvei*, each larvae receiving approximately $8{\cdot}3 \times 10^7$ spores. The comb containing them was incubated as in previous experiments. At the conclusion of this time a very large proportion of the larvae were full of sporulating cells of *B. alvei*, though a few had apparently resisted the organism, and appeared sterile on ordinary microscopical examination. The comb containing these artificially infected larvae was then suspended in the nucleus.

Date of inoculation. 25 June.

Results. 2 *days*: Most, but not all, of the slimy larvae in the introduced comb had been removed by the bees, and eggs laid in the empty cells. 5 *days*: All the inoculated larvae removed and no sign of infected larvae in any of the combs. 7 *days*: Young larvae being reared in the introduced comb, no sign of infection. 10 *and* 14 *days*: No sign of infection. 28 *days*: Fifty-one sick or dead larvae counted in the brood frames. Many of these were slimy and evil smelling. Ten were examined microscopically, and all showed *B. alvei* cells in large numbers, and the more recently infected larvae showed *S. apis* cells and a variable number of small rod-shaped bacteria. 32 *days*: The nucleus was destroyed, the queen being retained together with twelve worker bees for Exp. 31. Seventy-two sick or dead larvae were counted in one of the brood frames, and many of these were brown, slimy and evil smelling. Thus four slimy larvae of ten examined microscopically contained sporulating cells of *B. alvei* in apparently pure culture, the remainder having a large number of *B. alvei* and *S. apis* cells and a variable proportion of small rod-shaped bacteria. *S. apis* (culture 7) and *B. alvei* (culture 15) were isolated in pure culture from remains of dead larvae.

Exp. 13.

Nucleus. Sealed brood; eggs and young larvae; young larvae (inoculated comb); sealed brood; sealed brood.

Inoculum. 1000 and 900 3–4-day-old larvae on either side of a comb were fed 0·0025 ml. of a spore suspension of *B. alvei*, each larva receiving about $1 \cdot 7 \times 10^7$ spores. The comb was then incubated as usual. At the end of this time many larvae were affected with the artificially induced disease and the comb containing them was inserted in the nucleus.

Date of inoculation. 9 July.

Results. 2 *days*: All infected larvae had been removed by the bees and eggs laid in the empty cells. Examinations 5, 7, 13 and 21 days after inoculation revealed no infection. 24 *days:* One suspicious-looking larva full of *S. apis* cells, and from this a pure culture (culture 3) of *S. apis* was isolated. Further examination of the brood 28, 32, and 34 days after inoculation revealed no infected larvae, and the experiment was concluded, since the nucleus had become queenless.

It is evident from the results of Exps. 12 and 13 that "European foul brood" can be initiated by placing a large number of larvae artificially infected with *B. alvei* in a healthy nucleus, provided this is done early in the brood-rearing season and that the nucleus is weak in bees. If the inoculation is carried out late in the brood-rearing season the bees are able to keep the disease in check. In the first experiment a "mixed infection" of the brood similar to "European foul brood" resulted when *B. alvei* alone was employed to infect the starved larvae. However, the relative proportion of *B. alvei* cells present in decaying larvae was much greater than in the experiment in which disease was induced employing a pure culture of *S. apis* (Exp. 11).

Exp. 14.

Nucleus. Sealed brood; eggs and young larvae; young larvae (inoculated comb); sealed brood. Strong in bees.

Inoculum. 720 and 945 2–4-day-old larvae on either side of a comb were fed 0·0025 ml. of a suspension containing a mixture of *S. apis* cells and *B. alvei* spores, each larva receiving approximately $1 \cdot 4 \times 10^6$ spores of *B. alvei* and $1 \cdot 6 \times 10^6$ vegetative cells of *S. apis*. The comb containing the inoculated larvae was incubated as usual. At the conclusion of the incubation period a large number of larvae had been attacked by the bacteria, and microscopical examination showed masses of *S. apis* cells and

B. alvei vegetative cells and spores in these. Some of the larvae were rather slimy, others were of a pasty consistency. The infected comb was then inserted in the nucleus.

Date of inoculation. 8 July.

Results. 2 *days*: All infected larvae had been removed from the introduced comb and eggs laid in the empty cells. 4 *days*: Eggs and healthy young coiled larvae in the introduced comb, no indication of disease. No infected larvae were observed during subsequent inspections of the brood 6, 7, 10, 11, 22, 25, 33, 35, 40 and 45 days after inoculation. By this time the nucleus was very strong in bees and breeding had almost ceased.

EXP. 15.

Nucleus. Stores and sealed brood; eggs and young larvae; young larvae (inoculated comb), eggs, young larvae and sealed brood. Very weak in bees.

Inoculum. 680 and 410 2–4-day-old larvae on either side of a comb were fed 0·0025 ml. of a mixed suspension of *S. apis* cells and *B. alvei* spores, each larva receiving approximately $9{\cdot}1 \times 10^7$ cells of *S. apis* and 5×10^7 spores of *B. alvei*. The inoculated larvae were starved in the comb as usual, and then introduced in the customary manner to the nucleus.

Date of inoculation. 21 July.

Results. 3 *days*: Most of the dead larvae had been removed by the bees from the inoculated comb, and eggs laid in the empty cells. No infected larvae seen. 5 *days*: One dead larva in a comb adjacent to the introduced comb, and this was full of *S. apis* and *B. alvei* cells. 9 *days*: No infected larvae seen. 16 *days*: Two dead larvae full of *S. apis* and *B. alvei* cells. *B. alvei* (culture 18) was isolated in pure culture from one of these larvae. 18 *and* 20 *days*: No sick or dead larvae were seen. 22 *days*: Two sick larvae full of *S. apis* cells and small rod-shaped bacteria. 27 *days*: Four larvae full of *S. apis* cells and small rod-shaped bacteria. *S. apis* (culture 10) was isolated from one of these larvae. 29 *days*: Six sick or dead larvae full of *S. apis* cells and small rod-shaped bacteria. 36 *and* 40 *days*: Breeding very slowly, and no sign of any infected larvae.

It is apparent from the results of these two experiments that healthy nuclei are no more liable to contract disease when a mixture of *S. apis* and *B. alvei* is employed to initiate disease than when only one of these organisms is used. It can also be seen that, while a nucleus weak in bees can contract a transient form of "European foul brood" late in the brood-rearing season by the means of inoculation employed, a strong nucleus will not contract the disease even though it be inoculated somewhat earlier in the brood-rearing season.

EXP. 16.

Nucleus. Sealed brood and stores; young larvae and sealed brood; young larvae (inoculated comb); young larvae and sealed brood.

Inoculum. Forty larvae sick or dead of "European foul brood" were triturated in 20 ml. of sterile water. 530 and 350 2–4-day-old larvae on either side of a comb were fed 0·0025 ml. of this suspension. The comb containing the inoculated larvae was incubated in the usual manner, with the result that a large number of the larvae died with masses of *S. apis* and *B. alvei* cells in their tissues, and in addition some small rod-shaped bacteria. The comb was then introduced into the nucleus.

Date of inoculation. 20 July.

Results. *2 days*: Most of the dead larvae in the introduced comb had been removed by the bees and eggs laid in the empty cells. No infected larvae were observed on examining the brood 4, 10, 13, 17, 21 and 23 days after inoculation. *28 days*: One sick larva full of *S. apis* cells recovered, and from this a pure culture of *S. apis* (culture 11) was isolated. Subsequent inspections of the brood 37 and 45 days after inoculation revealed no infected larvae.

From this experiment it would appear that crushed suspensions of naturally infected "European foul brood" larvae are no more capable of initiating serious infection in the brood of a healthy nucleus relatively late in the brood-rearing season than are pure cultures of *S. apis* and *B. alvei* when the same technique is employed.

Exp. 17.

Nucleus. Stores; eggs, young larvae and sealed brood; young larvae (inoculated comb); eggs and sealed brood.

Inoculum. A Pasteur-Chamberland L 3 filtrate from European foul brood material was obtained by the technique previously described, forty larvae in all stages of the disease being crushed in 20 ml. of water for the purpose. A portion of the resulting filtrate was mixed with an equal volume of a suspension containing *S. apis* cells and *B. alvei* spores. 900 larvae 2–4 days old on one side of the comb, and 700 the other side, were fed 0·0025 ml. of this suspension, each larva receiving 0·00125 ml. of filtrate, $8{\cdot}4 \times 10^7$ *S. apis* cells and $2{\cdot}6 \times 10^7$ spores of *B. alvei*. The inoculated comb was incubated in the usual manner, many of the larvae decomposing with masses of *S. apis* and *B. alvei* cells in their tissues. It was then suspended in the experimental nucleus.

Date of inoculation. 19 July.

Results. *3 days*: Nearly all the dead larvae in the inoculated comb had been removed by the bees, only a few brown scales remained. Eggs being laid in the empty cells. No infected larvae were seen during examinations of the brood 6, 11, 13, 18, 23, 28 and 37 days after inoculation. At the conclusion of this time, breeding was very slow.

This experiment shows that filtrate from infected brood mixed with the bacteria found in diseased larvae is no more capable of initiating disease relatively late in the brood-rearing season than are pure cultures of the bacteria alone. This may be looked upon as further evidence in favour of the fact that a virus is not implicated in the etiology of "European foul brood".

Exp. 18.

Nucleus. Young larvae and sealed brood; young larvae (starved without inoculation); eggs and young larvae; young larvae and sealed brood.

Inoculum. A comb containing a large number of 1–4-day-old larvae was selected, and was incubated as usual, the larvae not being inoculated. At the conclusion of the incubation period the comb contained many autolysing larvae, ten of which, on microscopical examination, appeared to be sterile. The comb was then inserted in the nucleus.

Date of inoculation. 27 June.

Results. *2 days*: Most of the starved larvae had been removed by the bees and eggs laid in the empty cells. Further observations 4, 7, 11, 20, 22, 30, 36, 44, 49, 50 and 63 days after inoculation revealed no infection.

EXP. 19.

Nucleus. Stores; eggs and young larvae; eggs and young larvae; young larvae (starved); eggs and young larvae; sealed brood and stores. Very weak in bees.

Inoculum. 475 and 550 2–4-day-old larvae on either side of a comb were each fed 0·005 ml. of sterile brood filtrate. The comb was then incubated for 4 days in the customary manner. Microscopical examination of six autolysing larvae from the comb at the end of this time revealed no bacteria. It was then inserted in the nucleus.

Date of inoculation. 24 July.

Results. The dead larvae were removed by the bees in the usual manner and no infected larvae were observed 6, 14, 19, 24, 30, 40 and 49 days after inoculation.

Exps. 18 and 19 may be regarded as control experiments, for they show that uninfected starved larvae will not induce infection in healthy nuclei early or late in the brood-rearing season.

EXP. 20.

Nucleus. Eggs, young larvae and sealed brood; eggs, young larvae and sealed brood; inoculated comb; sealed brood.

Inoculum. A comb of freshly drawn foundation was selected, and each of 600 empty cells near the centre of the comb received 0·005 ml. of a suspension containing approximately $6{\cdot}7 \times 10^9$ cells of *S. apis*. The comb was incubated 12 hours at 33° C., in order to dry the bacterial suspension, and it was then suspended in the nucleus.

Date of inoculation. 15 July.

Results. 10 *days*: A very even patch of sealed brood and young larvae had been reared by the bees on the inoculated side of the introduced comb, and no indication of any infection. No infected larvae were seen 15, 22, 26, 33 and 42 days after inoculation.

EXP. 21.

Nucleus. Stores; eggs and young larvae; inoculated comb; sealed brood; sealed brood and stores.

Inoculum. Approximately 2×10^8 spores of *B. alvei* were dried in each of 600 empty cells in a brood comb as in the previous experiment. The comb was then introduced into the nucleus.

Date of inoculation. 15 July.

Results. Eggs and young larvae almost ready for sealing were present in the inoculated comb and no infected larvae were seen. Examinations of the brood 15, 23, 28, 33 and 40 days after inoculation revealed no infected larvae.

From the results of the above two experiments it would appear that it is not possible to infect healthy nuclei relatively late in the brood-rearing season by the method employed.

EXP. 22.

(*a*) *Nucleus.* Stores; eggs and young larvae; eggs; young larvae and sealed brood; sealed brood and stores. Breeding relatively slowly.

Inoculum. *S. apis* was inoculated into 200 ml. of sterile milk containing 10 ml. of brood filtrate, and the resulting culture incubated for 12 hours at 35° C. The resulting coagulated milk was poured into the empty cells of the brood combs of the nucleus, some of it being poured over the coiled larvae.

Date of inoculation. 7 August.

Results. 3 *days*: Two suspicious-looking larvae; both of these contained a few *S. apis* cells. 5 *days*: No sign of infection. 10 *days*: One larva still living contained a few *S. apis* cells and small rod-shaped bacteria. No infected larvae were seen 19, 26 and 34 days after inoculation.

(*b*) *Nucleus.* Stores; eggs and sealed brood; eggs and young larvae; eggs and sealed brood; sealed brood and stores.

Inoculum. The method of inoculation was identical with that given above except that the milk culture was heated at 100° C. for 30 min. in order to kill the bacteria.

Date of inoculation. 7 August.

Results. No infected larvae were seen during examinations of the brood at the same intervals as recorded in the above experiment. This experiment was the control.

Exp. 23.

(*a*) *Nucleus.* Stores and sealed brood; eggs and young larvae; eggs and young larvae; eggs and stores. Breeding relatively slowly.

Inoculum. *B. alvei* was cultivated for 7 days at 35° C. in 250 ml. of milk containing 10 ml. of brood filtrate. At the end of this time 88×10^9 spores of *B. alvei* in 10 ml. of suspension were added to the milk culture in order to increase the inoculum. The culture was then poured over the brood frames of the nucleus.

Date of inoculation. 8 August.

Results. Examinations 2, 4, 9, 18, 24, 28 and 36 days after inoculation revealed no infected larvae.

(*b*) *Nucleus.* Sealed brood and stores; eggs and young larvae; young larvae; stores. Breeding relatively slowly.

Inoculum. The inoculum was identical with that employed in the first part of the experiment except that the milk culture was autoclaved in order to kill the spores of *B. alvei*.

Date of inoculation. 8 August.

Results. No infected larvae were seen at intervals of examination identical with those employed in part (*a*) of the experiment.

The results of Exps. 22 and 23 indicate that it is not possible to infect healthy nuclei with milk cultures of *S. apis* or *B. alvei* late in the brood-rearing season. The experiments should be repeated early in the brood-rearing season.

Exp. 24.

Nucleus. Stores; sealed brood and young larvae; eggs and young larvae (inoculated); eggs and stores; sealed brood.

Inoculum. 50 ml. of a suspension of *S. apis* cells containing approximately $1{\cdot}4 \times 10^{13}$ organisms was poured over the eggs and young larvae of one of the brood combs. The comb was then suspended in the nucleus.

Date of inoculation. 22 July.

Results. 3 *days*: A fairly large number of the larvae in the inoculated comb had been removed by the bees as was evidenced by a pronounced irregularity in distribution of the brood. Further examinations 8, 11, 16, 21, 26, 35 and 42 days after inoculation revealed no infected larvae.

This experiment indicates that disease cannot be induced in a healthy nucleus fairly late in the brood-rearing season by pouring relatively large numbers of *S. apis* vegetative cells over young coiled larvae.

II. *Inoculation of experimental nuclei by feeding the bees suspensions containing bacteria or other infective material*

Exp. 25.

Nucleus. Stores; sealed brood; eggs; young larvae and eggs.

Inoculum. Forty larvae sick or dead of "European foul brood" were ground up with 50 ml. of sterile syrup and fed to the bees of the nucleus.

Date of inoculation. 10 July.

Results. 2 *days*: All the infective material had been taken by the bees. 5 *days*: Three sick larvae seen. 9 *days*: Four sick larvae in one comb. All of these had *S. apis* cells in large numbers, and two of them small rod-shaped bacteria as well. 13 *days*: Six affected larvae were recovered. All of these contained large numbers of *S. apis* cells and a variable proportion of small rod-shaped bacteria. *B. alvei* cells were seen in one of the affected larvae. Pure cultures of *S. apis* (culture 5) and of *B. alvei* (culture 13) were isolated. 22 *days:* Four larvae full of *S. apis* cells and small rod-shaped bacteria were recovered. One of these contained a fair number of *B. alvei* cells. 33 *days*: One affected larva full of *S. apis* cells recovered. Subsequent examinations of the brood 45, 51, 55 and 62 days after inoculation revealed no further infection.

This experiment is of interest in that it shows that a nucleus of hybrid bees is capable of keeping "European foul brood" in check, even when a relatively large inoculum of infectious material from natural sources is given, providing that it is given at a time when brood rearing is about to decline.

Exp. 26.

Nucleus. Eggs; young larvae; stores. Weak in bees.

Inoculum. The bees were fed infectious material on four successive occasions as follows:

1st day: 34×10^{10} vegetative cells of *S. apis* is 50 ml. of syrup.
3rd day: 44×10^{10} ,, ,, ,, ,,
5th day: 52×10^{10} ,, ,, ,, ,,
19th day: 46×10^{10} ,, ,, ,, ,,

Date of first inoculation. 8 July.

Results. Inspections of the brood up to the time of the last inoculation, and thereafter at intervals of 28, 36, 40, 47, 56 and 66 days after the first inoculation, revealed no infected larvae.

Exp. 27.

Nucleus. Eggs; young larvae; stores. Weak in bees.

Inoculum. The bees were fed infectious material on three successive occasions as follows:

1st day: 4×10^{11} spores of *B. alvei* in 50 ml. of syrup
8th day: $4{\cdot}6 \times 10^{11}$,, ,, ,,
10th day: $5{\cdot}1 \times 10^{11}$,, ,, ,,

Date of first inoculation. 2 July.

Results. No infected larvae were observed up to the time of the last feeding, nor at intervals of 16, 23, 32, 36, 41, 46 and 64 days after the first feeding.

From the results of these experiments it would seem that nuclei are not infected by feeding the bees large numbers of *S. apis* vegetative cells or *B. alvei* spores, even when several successive doses are fed.

Exp. 28.

Nucleus. Sealed brood and stores; eggs, young larvae and sealed brood; drawn comb; eggs, young larvae and sealed brood; stores and sealed brood.

Inoculum. 20 ml. of a Pasteur-Chamberland L 3 filtrate prepared in the usual manner from 100 larvae sick or dead of "European foul brood" crushed in 30 ml. of sterile brood filtrate were fed to the bees of the nucleus.

Date of inoculation. 19 July.

Results. No infection was observed 3, 6, 11, 22, 29, 37 and 45 days after inoculation.

Exp. 29.

Nucleus. Eggs and young larvae; sealed brood and stores; sealed brood and stores; eggs and young larvae; sealed brood and stores.

Inoculum. 25 ml. of a Pasteur-Chamberland L 2 filtrate prepared in the usual manner from ninety "European foul brood" larvae in all stages of disease crushed in 50 ml. of sterile brood filtrate were fed to the bees of the nucleus.

Date of inoculation. 22 July.

Results. No infected larvae were seen 3, 9, 15, 21, 26, 33 and 42 days after inoculation.

Exp. 30.

Nucleus. Stores; eggs and young larvae; sealed brood; stores; eggs and young larvae.

Inoculum. The bees were fed infectious material on each of two successive days as follows:

First feeding: 2×10^{10} spores of *B. alvei*; $6{\cdot}7 \times 10^{11}$ vegetative cells of *S. apis*; 5 ml. of a Pasteur-Chamberland L 2 filtrate, prepared as in Exp. 29; and 15 ml. of sterile syrup.

Second feeding: 2×10^{11} spores of *B. alvei*; $3{\cdot}7 \times 10^{11}$ vegetative cells of *S. apis*; 5 ml. of the Pasteur-Chamberland L 2 filtrate; and 15 ml. of sterile syrup.

Date of inoculation. 15 July.

Results. No infected larvae were found when the brood was examined 4, 8, 18, 24, 33, 35, 40 and 43 days after inoculation.

The above three experiments may be regarded as additional evidence in favour of the fact that a virus is not implicated in the etiology of "European foul brood".

III. *The results of transferring queens from nuclei affected with "European foul brood" to healthy queenless nuclei*

Exp. 31.

The queens from the nuclei used in Exps. 2, 10 and 12 were kept for 48 hours in queen cages with twelve worker bees; the cages containing candy as usual. Three three-comb nuclei (two combs of sealed brood and stores and one frame with foundation) were made up, and one of the above queen bees was introduced into each nucleus, the date of introduction being 1 or 2 August.

Results. 9 *days* after introduction: Large numbers of eggs had been laid in at least two frames of each nucleus. 16 *days*: Eggs, young larvae and sealed brood in each nucleus, and no sign of any infected larvae. Further inspections of the brood 23, 29 and 41 days after the introduction of the queens revealed no infected larvae.

This experiment may be regarded as further evidence in favour of the fact that "European foul brood" is not carried by the queen.

DISCUSSION

It is evident from the results of the experiments described in this paper that "European foul brood" can be initiated in healthy nuclei by feeding either the bees or the larvae direct naturally infected material. However, the success attendant upon such experiments varies greatly according to the conditions under which they are carried out. Thus disease was readily induced in a nucleus weak in bees in the active part of the brood-rearing season by inserting in it a comb containing many larvae affected with "European foul brood". When small amounts of a suspension of crushed "European foul brood" larvae were fed direct to larvae rather early in the brood-rearing season different results were obtained in each of two experiments. In one case the disease appeared but was merely of a mild transient form, never assuming serious proportions, and disappearing toward the end of the brood-rearing season. In the other case no disease resulted; probably because the nucleus was strong in bees of a good "house-cleaning" type. When forty larvae affected with "European foul brood" were fed in syrup to the bees of a healthy nucleus about half-way through the brood-rearing season, disease resulted but was not of a serious kind, and toward the end of the brood-rearing season no sick or dead larvae could be found. In a further experiment small amounts of crushed "European foul brood" larvae were fed directly to young coiled larvae, the comb containing them being incubated for 4 days at 35° C. The comb containing these was then introduced into a healthy nucleus rather late in the brood-rearing season, with the result that no disease developed.

Experiments have also shown that "European foul brood" can be induced in healthy nuclei by inserting in them combs containing larvae which have been artificially infected by feeding them pure cultures of *B. alvei* or *S. apis* and starving them for 4 days at 35° C. The susceptibility of the larvae of healthy nuclei to infection when combs containing larvae thus infected are inserted in them appears to be governed by approximately the same conditions as pertain to inoculations made with infected larvae obtained from natural sources. Thus, in the experiments recorded, inoculation of nuclei by this means early in the brood-rearing season was followed by a severe infection, while later in the season either a very mild transient infection resulted, or no disease was produced. It is of some interest that even when a pure culture of *S. apis* or *B. alvei* was used to infect the larvae which were employed in the starvation experiments the disease which ultimately resulted was a mixed one in which both these bacteria were recovered from certain of the infected larvae. However, the relative proportion of *B. alvei* and *S. apis* varied, depending on which of them had been employed in the original inoculations. Thus, in the case of an experiment made using starved larvae which had been infected with *S. apis*, the number of larvae with masses of sporulating *B. alvei* cells was less than in the case of a similar experiment in which *B. alvei* had been employed to inoculate the larvae prior to starvation. In further experiments a mixture of *B. alvei* and *S. apis* was fed to larvae which were subsequently starved in their combs and then introduced into healthy nuclei. In one experiment a mild transient form of "European foul brood" resulted when a nucleus very weak in bees was inoculated in this manner quite late in the brood-rearing season. In another experiment in which a nucleus of normal strength was inoculated much earlier in the brood-rearing season no disease resulted. Certainly it would appear from these results that a mixture of *S. apis* and *B. alvei* cells is no more capable of initiating disease than one of these organisms alone by the technique employed. Control experiments in which uninfected starved larvae were introduced into nuclei early and late in the brood-rearing season were unsuccessful as regards initiating disease.

It is of some interest that a mixed bacterial infection of the brood ultimately resulted when pure cultures of *B. alvei* or *S. apis* were used to infect larvae by the "starved larvae" technique. The reason for this is not known. It does seem possible that the bees introduce bacteria into recently infected or decaying larvae. Certainly bees are by no means sterile creatures bacteriologically, and Bruce White(22) found that normal healthy bees carry an organism similar to *S. apis* in their intestinal tracts. It is well known that the normal hive contains certain spore-forming bacteria, including *B. alvei*. In general the results of these experiments suggest that "European foul brood" may not be a single disease, but is, perhaps, a mixed bacterial infection of the brood of weak colonies of bees. However, any absolute conclusion regarding this must be postponed until additional experiments can be carried out.

Up to the present time it has not been found possible to infect the brood of healthy nuclei by feeding the bees several successive doses containing either *S. apis* vegetative cells or *B. alvei* spores in large numbers. Likewise no disease has been initiated when larvae have been fed these organisms directly and then immediately placed in healthy nuclei without a preliminary starvation period. In this respect the results obtained by Borchert (5, 6) have not been verified. The reason for this is not clear, but it is possible that the race of bee used by Borchert was one which was very susceptible to disease of the "European foul brood" type. Attempts to cause infection by pouring milk cultures *S. apis* or *B. alvei* over developing brood of healthy nuclei have also proved unsuccessful; however, these experiments have been carried out rather late in the brood-rearing season. Bacteria-free filtrates prepared by filtering crushed suspensions of "European foul brood" larvae through Pasteur-Chamberland L 2 or L 3 filters have not caused infection when fed directly to the larvae or to the bees of healthy nuclei, either alone or in combination with bacteria. From these results it would appear permissible to conclude that a filterable virus is in no way implicated in the etiology of this type of brood infection, especially in view of the successful results obtained with pure cultures of bacteria.

The results of all the experiments outlined above indicate that, with the type of bee used, it is normally not easy to initiate "European foul brood" in healthy nuclei unless inoculations are made early in the brood-rearing season, that is at a time when the preponderance of young larvae over both "nurse" and "house-cleaning" bees is relatively high, and consequently at a time when the larvae may be subject to a condition approaching malnutrition. This finding is in direct agreement with the important observations made by Sturtevant (13) with reference to the conditions favouring the spread of "European foul brood". This investigator found that black and hybrid bees are more susceptible to "European foul brood" than are Italian races; that the disease normally occurs only during the early part of the brood-rearing season; that Italian stocks of normal strength will not contract the disease; that the disease frequently disappears when an affected stock experiences a good honey flow and the season advances; and that it is relatively easy to infect a weak stock of black or hybrid bees in the spring, but that it becomes increasingly difficult, if not impossible, to infect such a stock during a heavy honey flow late in the summer when breeding is slow.

At present no really satisfactory explanation can be advanced for the fact that such a large inoculum of infected starved larvae is required to induce disease, and that bacteria fed directly to bees or to larvae which are subsequently attended to by the "nurse bees" have not, in the experiments recorded in this paper, caused disease. Perhaps a considerable attenuation with respect to virulence follows when the bacteria are cultivated for even a short time upon laboratory media, and passage through larvae whose resistance has been weakened by starvation may restore virulence. Again the possible influence of decomposing brood as a convenient "vehicle" for transmitting the disease must be considered, as well as the fact that the initial inoculum is probably greater when the "starved larvae" technique is employed than when bacterial suspensions are fed.

The cause of so-called "European foul brood" has long been a matter of controversy. White (19, 20) believed that a microorganism, *B. pluton*, which could not be cultivated on any medium studied caused this disease. Lochhead (9, 10) suggested that *B. pluton* did not exist as an organism distinct from *S. apis*, and that the forms seen by White, and which were called *B. pluton* by him, were merely lanceolate-shaped vegetative cells of *S. apis*. He also concluded, on the basis of certain morphological observations, that "European foul brood" might be caused by a pleomorphic organism which could assume the form of *S. apis* or *B. alvei*. This view has recently been adopted by Burnside (7, 8), who apparently believes that *S. apis*, *B. alvei* and *Bacterium eurydice* may all be forms of a single pleomorphic organism. Since this worker has also suggested that so-called *B. para-alvei* may dissociate into *S. apis* one is forced to conclude, on this theory, that all four organisms are pleomorphic forms of a single organism. While this view has not been disproved as yet the evidence advanced in favour of it is incomplete, and there is room for further investigation. Borchert (1, 2, 3, 4, 5, 6) has recently advanced certain evidence in favour of the fact that so-called "European foul brood" is not a single disease, but merely represents a mixed bacterial infection of the brood. This explanation seems a fairly plausible one in view of the facts already recorded in this paper. Moreover, the disease varies considerably as regards bacterial flora. Thus several different species of spore-forming bacilli have been described as occurring in different cases of this type of infection (5, 6, 16). Also mixed bacterial diseases of the brood other than the disease described as "European foul brood" by White (19, 20) have been noticed (8, 17, 18).

The results obtained by certain other investigators (3, 4, 5, 7, 9, 10) have led them to assume that *B. fluton* White does not exist as a species distinct from *S. apis*, but perhaps this conclusion should not yet be regarded as final. Certainly the lanceolate-shaped coccus cells seen in larvae affected with "European foul brood" are apparently not very different from those encountered in pure cultures of *S. apis* or those seen in larvae artificially infected with this organism employing the "starvation technique" (Pl. XXII, fig. 6 and Pl. XXIII, figs. 7–12). It is not unlikely that so-called "European foul brood" is no well-defined single disease. Rather would it appear that it may be a mixed bacterial infection of the brood of bees occurring in the main in the brood of weak colonies, and that it has no single specific etiological agent. The theory that the bacteria found in "European foul brood" are pleomorphic forms of a single species must be regarded with caution until more experimental work has been carried out. As yet the evidence presented in support of this hypothesis is by no means adequate enough to warrant its adoption.

Summary

"European foul brood" has been initiated in healthy nuclei by feeding either the young larvae directly or the bees naturally infected material. The disease thus produced varied from a mild transient infection to a serious form depending upon whether the inoculation had been made early or late in the brood-rearing season. In one experiment in which a nucleus very strong in bees was employed no disease resulted.

"European foul brood" has also been induced in healthy nuclei by suspending in them combs containing artificially infected larvae in which disease had been produced by feeding them pure cultures of *Streptococcus apis* or *Bacillus alvei* and then starving them under conditions favouring the growth of the bacteria. The susceptibility of nuclei to infection by this means seems to be governed by approximately the same conditions as pertain to inoculations made with material obtained from natural sources. The disease thus induced ultimately becomes a mixed bacterial infection of the brood.

Attempts to cause disease by feeding the bees or larvae (without starving them) relatively large numbers of *S. apis* or *B. alvei* organisms have as yet proved unsuccessful. Whether this is due to the fact that these bacteria become attenuated with respect to virulence by culturing them on artificial media, or that decomposing brood acts as a vehicle and that the relative inoculum is greater by this method, remains to be determined.

The failure to produce "European foul brood" by feeding sterile Pasteur-Chamberland L 2 or L 3 filtrates prepared from naturally infected larvae to the bees or larvae of healthy nuclei, either with or without bacteria, may be taken as strong evidence in support of the

view that a filterable virus is in no way implicated as an etiological agent in this type of disease. This conclusion is strengthened by the success which has attended the use of pure cultures of the bacteria associated with this disease employing the "starved larvae" technique described.

The introduction of queen bees from nuclei affected with "European foul brood" into healthy queenless nuclei has not caused any transmission of the disease under the conditions of the experiments.

Two species of *S. apis* Maassen have been isolated from affected larvae taken from several different cases of "European foul brood"; one of these hydrolyses both casein and gelatine, the other does not. In other respects these species appear to be identical.

The etiology of so-called "European foul brood" is discussed in detail, and it is suggested from the evidence submitted in this and other papers that it may not be a single disease with one well-defined etiological agent, as is American foul brood, but is, perhaps, a non-specific mixed bacterial infection of the brood of bees, especially of the brood of weak colonies. This conclusion must be regarded as temporary pending further investigation.

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EXPLANATION OF PLATES XXII–XXIV

(All photographs × 1200)

PLATE XXII

Fig. 1. *Streptococcus apis* (culture 1) after 16 hours' growth on beef-digest agar. This strain was a gelatin and casein hydrolyser and was employed in all the infection experiments.

Fig. 2. *Streptococcus apis* (culture 3) after 24 hours' growth on beef-digest agar. Note the chain of cells. This strain was not a casein or gelatin hydrolyser.

Fig. 3. *Streptococcus apis* (culture 3) after 24 hours' growth on Lochhead's sucrose-glucose-peptone agar (10).

Fig. 4. *Streptococcus apis* (culture 1) after 16 hours' growth on Lochhead's sucrose-glucose-peptone agar (10).

Fig. 5. *Streptococcus apis* (culture 1) after 12 hours' growth on beef-digest agar containing 1 ml. of $M/5$ phosphate buffer pH 7·0 per 5 ml. Note the very variable size of the cells.

Fig. 6. *Streptococcus apis* growing in the tissues of a starved larva after 4 days at 35° C. Note the lanceolate-shaped cells.

PLATE XXIII

Fig. 7. *Streptococcus apis* and *Bacillus alvei* (a single vegetative cell) growing in a larva sick of "European foul brood".

Fig. 8. *Streptococcus apis* showing a mass of typical lanceolate-shaped cells in a larva affected with "European foul brood". A few small rod-shaped bacteria (*Bacterium eurydice?*) can also be seen.

Fig. 9. *Streptococcus apis* and *Bacillus alvei* growing in the tissues of a larva which had been fed a mixed suspension of the cells of both these organisms and then starved for 4 days at 35° C.

Fig. 10. *Streptococcus apis* and *Bacillus alvei* growing in the tissues of a "starved larva".

Fig. 11. *Streptococcus apis, Bacillus alvei* and a few small rod-shaped bacteria (*Bacterium eurydice?*) growing in the tissues of a larva affected with "European foul brood". This larva was one of those recovered in Exp. 15.

Fig. 12. A preparation similar to that shown in Fig. 11, only from another case of "European foul brood".

PLATE XXIV

Fig. 13. *Bacillus alvei* growing in a "starved larva". This larva was brown, slimy and rather evil smelling as a naturally infected larva. Note the spore formation.

Fig. 14. *Bacillus alvei* in a slimy decaying "European foul brood" larva. The sporulating vegetative cells arranged round the large fat body are similar to those shown in Fig. 13.

Fig. 15. *Bacillus alvei* and *Streptococcus apis* in a larva dead of "European foul brood".

Fig. 16. *Streptococcus apis* and a torula or yeast in a larva dead of an atypical "European foul brood". In this case the torula or yeast appeared to take the place normally occupied by *Bacillus alvei* (17).

Note. In most of the preparations made from affected larvae a greater or lesser number of round fat bodies of variable size are to be seen; e.g. Figs. 6, 8, 9, 10, 12 and 13.

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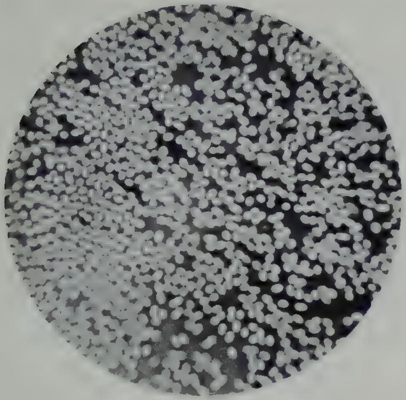

Fig. 1

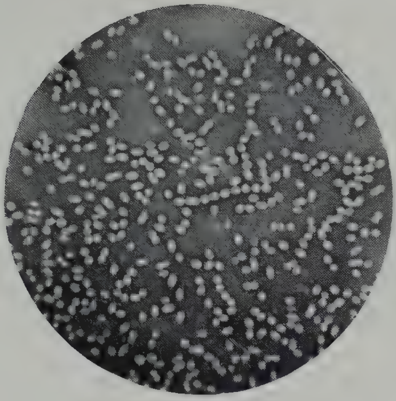

Fig. 2

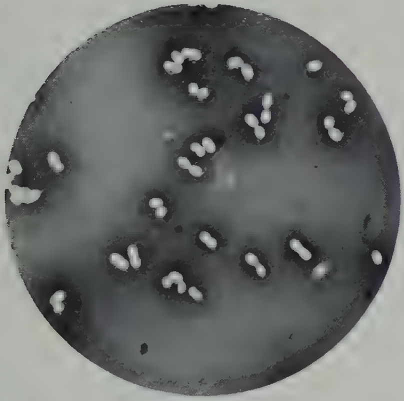

Fig. 3

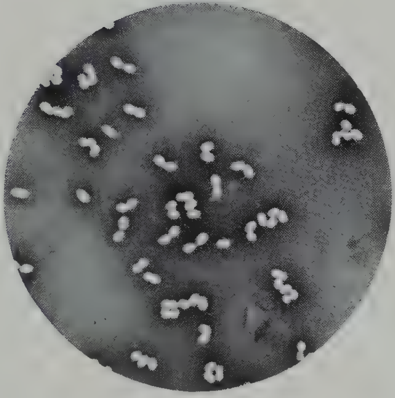

Fig. 4

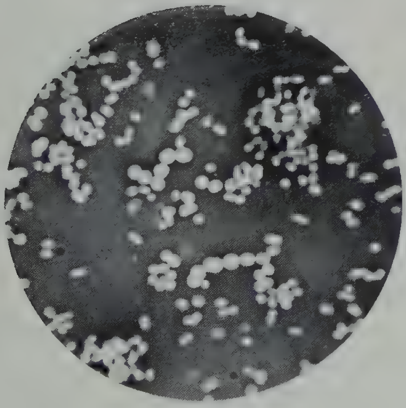

Fig. 5

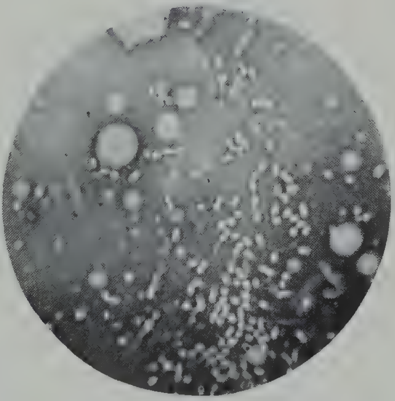

Fig. 6

TARR—Studies on European Foul Brood of Bees (pp. 558–584)

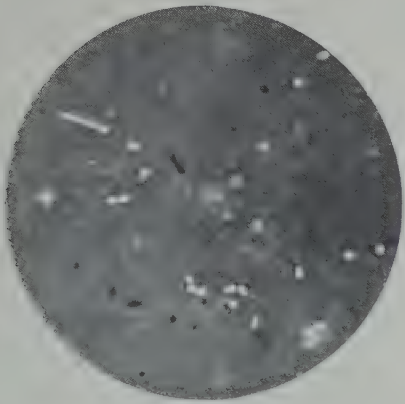

Fig. 7

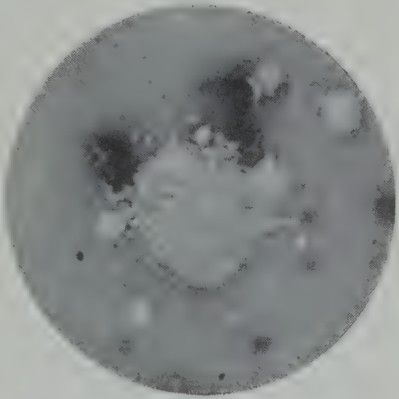

Fig. 8

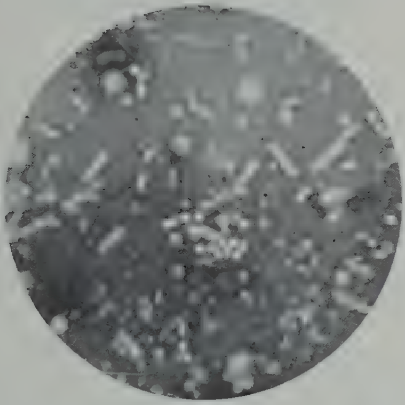

Fig. 9

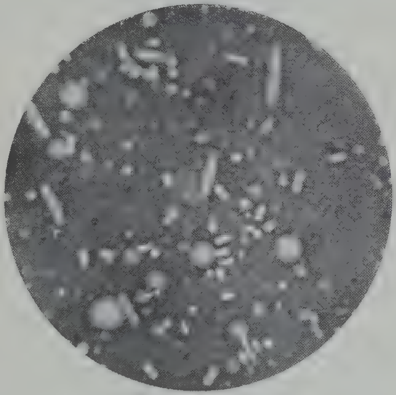

Fig. 10

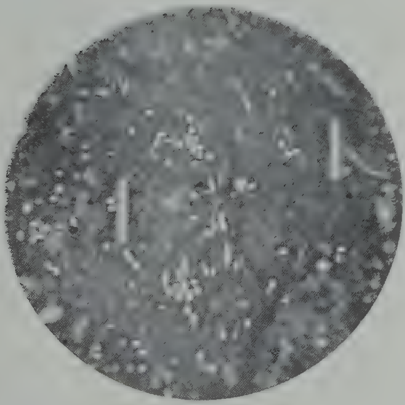

Fig. 11

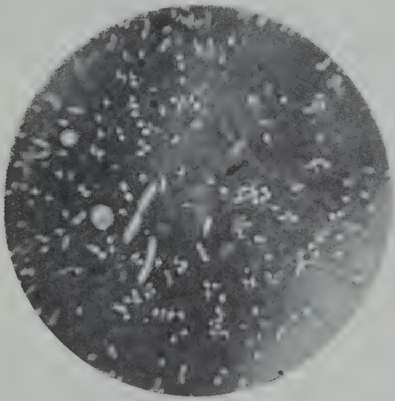

Fig. 12

TARR—Studies on European Foul Brood of Bees (pp. 558–584)

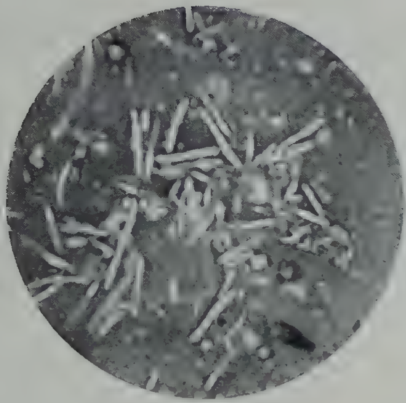

Fig. 13

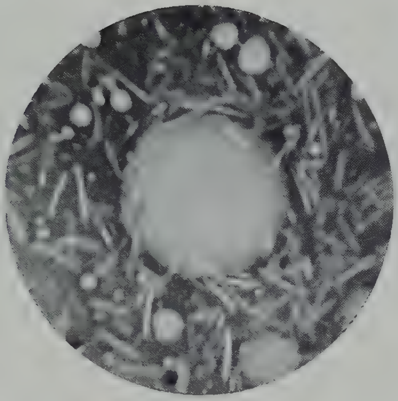

Fig. 14

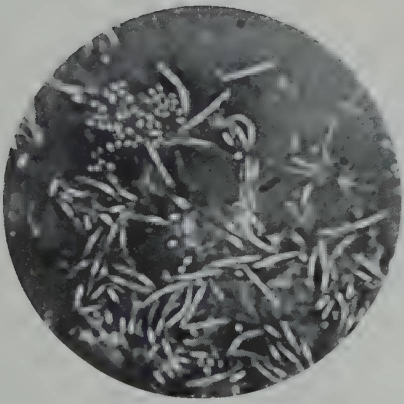

Fig. 15

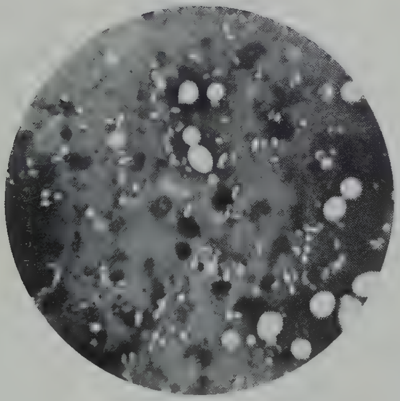

Fig. 16

TARR—Studies on European Foul Brood of Bees (pp. 558–584)

ADELGES COOLEYI GILLETTE (HEMIPTERA, ADELGIDAE) OF THE DOUGLAS FIR IN BRITAIN: COMPLETION OF ITS LIFE CYCLE

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(With Plate XXV and 3 Text-figures)

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I. INTRODUCTION

As remarked by Laing (5), the name *Chermes*, rendered familiar to students of Forest Entomology by long usage, has been superseded by *Adelges* for the reason that the former is preoccupied by a genus of the family Psyllidae, jumping plant-lice. Consequently, the family Chermesidae is now known as the Adelgidae.

It is not the purpose of the author to review in detail the complex life cycle of *Adelges cooleyi*, but to present an account of his recent investigations, which serve to reduce the gaps in existing knowledge of this important pest of Douglas fir and Sitka spruce in Britain. Indigenous to North America and first recorded in Britain in 1913, it is considered to have been introduced into the latter with nursery stock at some earlier date. However this may be, the insect is now thoroughly well established, and its distribution extends from the south and west of England to Ross and Cromarty in Scotland. In Ireland it has been recorded from the north-east.

For permission to conduct my observations of *A. cooleyi* in the forest at Glen Tress, Peeblesshire, I am indebted to the Forestry Commission. Likewise, to various private owners of plantations of Douglas fir and Sitka spruce, I wish to acknowledge the courtesy of their estates.

II. GENERATIONS OF THE ADELGIDAE

(1) *Alternate hosts*

Thanks to the researches of Chrystal (2, 3), who had the opportunity of studying *A. cooleyi* first in Canada at Stanley Park, Vancouver, and later in England and Scotland, the salient facts of its intricate life cycle were made available and its alternation of hosts established. For the sake of clarity, it may be recalled that a typical life cycle of the Adelgidae involves five distinct generations on two coniferous hosts, of which the primary is invariably a species of *Picea* and the secondary, one or other species of *Pinus*, *Larix*, *Abies*, *Pseudotsuga* and *Tsuga*, the selection of the latter depending upon the particular species of adelgid concerned. In certain species of Adelgidae the life cycle may be abbreviated by the omission of one or more of the five generations, and the partial cycle may be confined in some to the primary, in others to the secondary host. So far as *Adelges cooleyi* is concerned, the life cycle conforms to the type of five generations, of which three are bred on spruce and the remaining two on Douglas fir. In Britain the chief primary host is *Picea sitchensis* Carr., the Sitka spruce, but to this species there must now be added

P. alba Link., the white spruce, on which the galls were found at Melrose (*vide* p. 590). So far as the secondary host is concerned, the green Douglas fir, *Pseudotsuga douglasii* Carr., is highly susceptible to attack, whilst the blue Douglas fir, *P. douglasii* var. *glauca* Sudw., is practically immune in Britain.

(2) *Differentiation of generations and outline of life cycle*

For convenience of reference, the designations of the five generations of *Adelges cooleyi*, applicable to those of the Adelgidae in general, together with the more important facts of their biology and habits in Scotland, have been concisely presented in Table I (p. 588). To these reference will later be made in discussing the individual generations. Meanwhile it will be noted that the gallicolae and sexuparae agree with the sexuales in having each five instars or one additional to those of the fundatrix vera and colonici. Adult gallicolae and sexuparae also agree in having five antennal segments, which contrast with the four of the sexuales and three of the fundatrix vera and colonici. Only adult gallicolae and sexuparae are winged, and, with the exception of the sexuales, all generations are parthenogenetic, males occurring but once in the biennial life cycle.

Hibernation is effected as first-stage nymphs of generations I and III. Migration from the primary to the secondary host is secured in late summer by winged gallicolae of generation II, which oviposit on the needles of the secondary host and thus introduce generation III. A reverse migration from the secondary to the primary host is made in early summer by winged sexuparae of generation IV, which oviposit on the needles of the latter and thus introduce generation V. That the two hosts may not be essential to the continued existence of all species of *Adelges* is well illustrated by *A. cooleyi*, which can breed indefinitely on Douglas fir without the intervention of the spruce generations.

III. HISTORICAL

(1) *America*

The accompanying diagrams (Text-figs. 1, 2) on page 598, borrowed from Chrystal (3), graphically represent the current idea of the life cycle of *A. cooleyi*, and, so far as I am aware, their content has neither been enlarged nor modified by any recent publication on the subject. In America (Text-fig. 1) the cycle is represented as being ostensibly complete, except that generation V was not observed. Nevertheless, its existence is *a priori*

Table I

Generations of Adelges cooleyi

Generation	Designation	Duration* of generation	No. of instars	No. of eggs per female	Period of incubation in days	Breeding host	Site of host	Stage of hibernation
I	Fundatrix vera (foundress, stem-mother): progeny of sexuales	July–May	4	300–500	7–10	Sitka spruce	Base of, or near, terminal bud	1st-instar nymph
II	Gallicolae (gall-dwellers): progeny of fundatrix vera	May–Sept.	5	100–150	7	Sitka spruce	Galled buds	—
III	Colonici (fundatrix spuria, stem-mother):							
	(1) Winter brood I (sistens):							
	(*a*) Progeny of gallicolae migrantes	Sept.–Apr.	4	40–60	22–27	Douglas fir	Needles	1st-instar nymph
	(*b*) Part progeny of previous winter brood I	June–Apr.						
	(*c*) Part progeny of summer brood I (progrediens I)	July–Apr.						
	(*d*) Progeny of summer brood II (progrediens II)	Aug.–Apr.						
	(2) Summer brood I: part progeny of winter brood I	May–June						
	(3) Summer brood II: part progeny of summer brood II	July–Aug.						
IV	Sexuparae (winged migrants): part progeny of winter brood I	Apr.–June	5	10–25	7–12	Douglas fir	Needles	—
V	Sexuales: progeny of sexuparae	June–July	5	1	?	Sitka spruce	Needles	—

* The duration of a generation is reckoned as extending from the time of oviposition of the antecedent generation to the time of death and disappearance of the adults which develop from the eggs thus laid. It covers the whole period of activity, not of individual members of a generation, but of a generation as a whole.

implied by the occurrence of generation I, which in the absence of non-migrating gallicolae, unknown in *A. cooleyi*, can only be inaugurated by pre-existing sexuales of generation I. In this regard it is interesting to learn that there is evidence, to which I will refer later (p. 603), that generation V now exists in British Columbia.

(2) *Britain*

In Britain, Chrystal (3) succeeded in finding the sexuales, but among the few adults of this generation that were discovered, only a solitary female was found. This default of the sexuales was to Chrystal the weak link in the chain of generations and the actual cause of the incomplete life cycle in Britain. Thus he concluded from the available facts that "the failure of the sexual generation precluded the possibility of gall formation, and prevented possibly serious injury being done to that tree". Hence the omission of generations I and II in Text-fig. 2; and that Chrystal found neither the fundatrix vera nor the galls on Sitka spruce in Britain would appear to be sufficiently explained by the impotence of generation V. In the light of these facts, the life cycle in Britain has been recognized as less complete than in America. In the former it has been hitherto considered as practically confined to the Douglas fir, where the damage caused by the feeding activities of generation III on the needles results in a retardation of growth, which affects young trees more seriously than older ones. The potentialities for injury to the host are, however, according to Chrystal's experience in Stanley Park, Vancouver, much greater in the case of the gallicolae on the Sitka spruce, in that the galled terminal buds are permanently destroyed and further growth does not occur distal to the gall. It was, therefore, considered a matter for congratulation in the interests of British forestry that the gall-forming generation had not become established in this country.

IV. *ADELGES COOLEYI* IN SCOTLAND, 1935; DISCOVERY OF GALLS IN BRITAIN

On 7 February I accompanied my colleague, Mr D. C. Fergusson, Lecturer in Forestry, University of Edinburgh, on an excursion of students of Forestry to the plantations of the Forestry Commission at Glen Tress, $1\frac{1}{2}$ miles east of Peebles. In the course of inspection Mr Fergusson discovered on the twigs of 10-year-old Sitka spruce, dead galls which were found to answer the description of those of *A. cooleyi* (Pl. XXV, fig. 3). On the same trees were dead galls of *A. abietis* and *Cn. strobilobius*, from which those of *A. cooleyi* are readily differentiated by their greater length, varying from 1½ to 3 in., and their almost uniform thickness. Later and more thorough examinations of the plantation revealed that the galls varied in age from 1 to 3 or 4 years. On young trees up to 10 years the galls were easy to find, being comparatively numerous on some, but rarer and more difficult to discern on older trees of 30 years, because of the greater height of the branches from the ground. Chrystal's (3) failure to find the galls of *A. cooleyi* in 1922, despite a comprehensive survey, which included nearly all parts of the country, makes it almost certain that they became established in Scotland sometime subsequent to his investigation, and possibly only in the last few years.

Time has not permitted extension of my investigations much beyond the confines of Glen Tress, but in the spring and summer of 1935 dead galls of *A. cooleyi* were found also at Melrose and Stobs, Roxburghshire; Galashiels, Selkirkshire; Dreghorn, Midlothian and Murthly, Perthshire. At Melrose they were comparatively common in a shelter-belt of white spruce, *Picea alba* Link, on the property of Dr W. B. R. Laidlaw, who suspected their identity in 1934. This discovery is of interest in that it is the second British record of the occurrence of *Adelges cooleyi* on a primary host other than Sitka spruce. The first was that of Laing (5) in 1929, who stated that so-called "woolly aphis" had been reported to the Forestry Department of Aberdeen University by an Aberdeenshire forester as having appeared and apparently become established on *Picea nigra* and *P. pungens* var. *glauca*, growing near infested Douglas fir. Laing identified the "woolly aphis" as *Adelges cooleyi*, but, unfortunately, he neglected to state the locality and date of its occurrence, or to which of the spruce generations his specimens belonged.[1] It is clear, however, from his account, that the galls were not discovered.

At Galashiels, old galls were found on Sitka spruce in a small, mixed, roadside plantation of this tree and Douglas fir. At Dreghorn near Edinburgh, little difficulty was experienced in finding the galls both in a lowland plantation at Hunter's Tryst and an upland one on the lower slopes of White Hill, where the trees had been planted 10–13 years. At Murthly they were recovered from Sitka spruce about 50 years old, but diligent search failed to reveal their presence in a neighbouring mixed plantation of Sitka spruce and Douglas fir established 21–30 years ago. Wherever the galls were found on the spruce, infested Douglas fir occurred in the immediate vicinity. So far, Norway spruce, *Picea*

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[1] In a letter dated 3 December 1935, Mr Laing informs me that the specimens submitted to him for identification were sexuparae and that they were collected at Dunecht, Aberdeenshire.

nordmannia, has yielded not a single gall of *Adelges cooleyi*. All the galls of *Adelges* which I have examined on this tree have proved to be either *A. abietis* or *Cnaphalodes strobilobius*.

Whilst the discovery of the old galls on Sitka spruce was itself of fundamental importance, it was felt that the enquiry should be enlarged to include the study of certain vexed problems of the life cycle that had not hitherto been satisfactorily solved. In particular, it was considered desirable to (1) prove the occurrence of generation I, fundatrix vera, on the spruce and follow its development; (2) trace the development of the gallicolae and investigate the process of gall formation; (3) observe the migration of the gallicolae from the spruce to Douglas fir and discover whether their progeny developed into sistentes on the latter host; (4) revise the cycle of the colonici generation on Douglas fir with a view to ascertaining the exact number of its component broods; (5) confirm the migration of the sexuparae from Douglas fir to Sitka spruce; (6) examine the probable causes of failure of the sexuales.

In the discussion which follows it will be convenient to deal with these questions in the order in which they are stated.

(1) *Generation I—fundatrix vera; first British record*

This generation, progeny of the sexuales, hibernates on the Sitka spruce as first nymphal instars, located in the crevices of the bark of the twigs, close to, or at a short distance from, the terminal bud. It has not hitherto been recorded in Britain. At Glen Tress on 12 April 1935, second- and third-stage nymphs were discovered, some of which, brought to the laboratory, completed their development. The first of the resulting stem-mothers or *foundresses* (Pl. XXV, fig. 1) commenced oviposition on 16 April, and under cover of a mass of enveloping, white, waxen wool, each laid a number of brown eggs varying from 300 to 500. In the laboratory, hatching commenced on 26 April; in the field, hatching was not observed until 9 May, when the buds were beginning to open and shed their investing scales. About a week later an unfortunate vagary of the weather seriously threatened the prospect of further development of the gallicolae. On the night of 16 May and early morning of 17 May an unseasonable frost of unusual severity occurred throughout the country. At Glen Tress, where a minimum temperature of 23° F. was recorded, it was computed that 75 per cent. of the buds, burst and unburst, had been irreparably damaged (Pl. XXV, figs. 1, 2). An examination of the buds was made on 22 May, when it was found that many of the unhatched egg masses of the stem-mothers had become blackened and had perished, and the first-instar gallicolae, which had previously migrated to the buds, had shared a similar fate. As a result, the number of potential galls was materially reduced and, with this, the opportunity to observe the development of the gall-forming generation in the field.

Despite the blighting effect of the frost a few undamaged egg masses of stem-mothers were found at Glen Tress as late as 28 May. The nymphs which hatched from these and likewise from the eggs that were collected previous to the onset of the frost provided specimens for the further investigation of the behaviour of the gallicolae in the laboratory.

(2) *Generation II—gallicolae; first British record*

(*a*) *Factors of gall formation; galls of* Adelges cooleyi; A. abietis *and* Cnaphalodes strobilobius *differentiated.*

It is a common opinion among students of the Adelgidae that gall formation in the spruce host is primarily induced by the feeding activities of the stem-mother or foundress of generation I situated at the base of the bud, and is later completed by those of her progeny within the bud. *Adelges cooleyi* differs from *A. abietis* and *Cnaphalodes strobilobius* in that the stem-mothers are not confined to the bases of the buds but may occur on the twigs at some distance from the terminal buds. If gall formation is assumed to be merely a reaction of the host in its efforts to repair the damage caused by the feeding of the insect, it is difficult to imagine how the stem-mother outside the bud can play an effective part in the process, especially if she is separated from the buds by a distance of 1 or 2 in.

Some investigators, however, maintain that a secretion introduced into the host tissues by the stem-mother whilst feeding retards the growth of the bud and stimulates the incipient development of the gall. If this were so, it would be natural to expect that all the buds which terminate a twig harbouring a stem-mother located at some distance from the buds would be equally affected by the secretion, since the latter would presumably be conveyed to the tissues of all the terminal buds by the vascular bundles. In *Adelges cooleyi* observation has shown that the facts are otherwise, and only those buds are galled which become infested by the first-stage gallicolae. Frequently the galled bud may be but one of a terminal group, the remaining non-infested ones developing in normal fashion.

What factor determines the choice of one bud rather than another by the young gallicolae, where several are available, has not been ascertained. When the first-stage nymphs have emerged from an egg mass laid by a stem-mother at the base of a bud, proximity to that bud may be the only determining factor of selection. On the other hand, two or more of a group of terminal buds were frequently found to be galled, where the stem-mother and her egg mass were situated well below the apex of the twig. Here the nymphs in travelling up the twig had become dispersed indiscriminately to various buds. The tendency for selection of the apical bud by the nymphs may be attributed to their marked negative geotropism, which induces them to maintain a migratory course to the apex of the shoot and tends to prevent their deviation to the lateral apical buds.

In the study of the gallicolae and their behaviour cut twigs of Sitka spruce with adherent stem-mothers of generation I (Pl. XXV, fig. 1) were collected at Glen Tress at various times between 16 April and 22 May and taken to the laboratory, where the twigs were placed with their cut ends in water. Such twigs were maintained alive for periods of 4–6 weeks, sufficiently long to observe the process of gall formation. Observations in the laboratory were supplemented by others made in the field at Glen Tress, when an incipient gall was first discovered on 28 May. From then onward, individual trees bearing galls were marked, so that particular galls could be kept under almost continuous observation during the summer.

The first-instar gallicolae on hatching from the eggs crawl from among the loosely felted, waxen fibres of the stem-mother and migrate to the opening bud. Passing down the exposed needles, they reach the central axis of the bud, along which they range themselves, in groups of six to ten, at the bases of the needles. Inserting their mouth stylets in the tender tissues, they begin feeding, with the result that the growth of the bud is retarded. The axis increases in length only slightly so that the leaves which become spaced in a normal shoot remain more or less contiguous at their bases. At the same time the bases of the needles undergo hypertrophy and fuse with adjacent ones, leaving only closed narrow interspaces or loculi, in which the young nymphs are ensconced. The aggregation of the feeding nymphs on the under surface of the axis causes a foreshortening of this surface, whilst the upper, if uninfested, continues its growth. The consequence of this differential growth is a curvature of the gall, in which the needles arising from the lower convex surface remain shorter than those from the upper. The gall (Pl. XXV, fig. 2) usually involves the whole length of the shoot from base to apex, so that there is no subsequent growth beyond the gall as in that of

A. abietis (Pl. XXV, fig. 7). Otherwise, the two types of gall are readily differentiated by their shapes. That of *A. abietis* is short and like a pine cone, whilst that of a third species, *Cnaphalodes strobilobius* (Pl. XXV, fig. 8), which also occurs on Sitka spruce, is small and stunted, with all the needles galled, and it terminates the growth of the shoot. It should be remarked that galls of *Adelges cooleyi* are frequently encountered which do not conform to type, in that the bud may be uniformly galled from base to apex and on all sides. Others again have been found where the shoot had continued to grow beyond the apex of the gall, and the continuation had been subsequently galled by *Cnaphalodes strobilobius* or *Adelges abietis*.

(*b*) *Changes in colour of gall during development*

In their development the galls of Adelgidae undergo striking changes of colour, which were particularly noted in individual galls of *Adelges cooleyi*, *A. abietis* and *Cnaphalodes strobilobius*, all on Sitka spruce. An incipient gall of *Adelges cooleyi* was observed to be entirely green on 24 May. On 27 May it revealed a delicate pink maculation or stippling, which in the next few days gradually deepened to purple. Concurrently with this change the individual maculae increased in extent and became coalescent, so that by 7 June the gall was uniformly deep purple in vivid contrast to the dark green of the projecting needles. The purple coloration persisted for 3 weeks, after which it gradually faded and was replaced by a bluish green (26 June). Subsequently the colour remained unchanged until opening commenced on 26 August to release the fourth-instar nymphs. Then followed desiccation and induration of the gall, accompanied by a final change of colour from green to the brown of the dead gall (Pl. XXV, fig. 3).

Similar changes of colour were observed in galls of *A. abietis* through green, stippled pink on green, diffused crimson, reddish purple and back to green. In the final green stage traces of purple still persist as narrow semicircular bands, one between each adjacent pair of swollen leaf bases, demarcating the future openings of the gall chambers.

In contrast to the first two, the gall of *Cnaphalodes strobilobius* is distinguished by the entire lack of rich pigmentary embellishment during its development. From its inception it has a blanched appearance, and varies between pale greenish white and pale yellow. The proportion, too, of the galled to the ungalled parts of the individual needles is much greater in the gall of *Cn. strobilobius* than it is in *Adelges abietis*, and least in *A. cooleyi*. This difference is readily recognized by a comparison of figs. 2, 7 and 8 of Plate XXV and is expressed by the greater length of the projecting needles in the gall of *A. cooleyi* than in that of *A. abietis*, those of *Cnapholodes strobilobius* being the shortest of the three.

(c) *Attempt to reproduce galls; localities where living galls were found*

Incidentally, it may be remarked that an attempt to induce reproduction of the galls of *Adelges cooleyi* on a young tree of Sitka spruce in the laboratory proved unsuccessful. In this experiment young trees of Sitka spruce were obtained from the nursery of the Forestry Department, University of Edinburgh, at Dreghorn, potted and taken to the laboratory on 22 April. Eggs of the fundatrix vera were collected at Glen Tress on 22 and 28 May and transferred to the bursting buds of the spruce in the laboratory. The experiment failed to produce results for two reasons. First, the percentage of eggs that hatched was negligible due to their previous exposure in the field to the destructive frost of 16 and 17 May, and second, because the buds of the spruce, to which they were transferred, had become too far advanced in their growth under laboratory conditions to be affected by the few first-stage nymphs that succeeded in hatching.

Whilst the investigations at Glen Tress and in the laboratory at Edinburgh required undivided attention, opportunity was made during the summer to pay a limited number of brief visits of inspection to other localities where combined plantations of Sitka or other spruce and Douglas fir were known to occur. These localities have already been cited (p. 590) in reference to the discovery of the dead galls of *A. cooleyi* of past seasons. In only two, other than Glen Tress, were the developing galls of this season found, at Murthly, 4 July and Dreghorn, 4 September, and they were comparatively rare. At the former it is interesting to record that the fresh galls occurred on trees of Sitka spruce 50 years old.

(d) *Dehiscence of gall, migration of gallicolae, oviposition*

In the laboratory, dehiscence of the galls was first noted on 17 August. Previously, on 12 August, a few ripe but unopened galls were dissected, and nymphs of the fourth instar, characterized by the possession of rudimentary wings, were disclosed in the gall chambers. It was not, however, until 23 August that the first voluntary emergence of the latter was observed in the laboratory. On the following day, several of the nymphs were seen to be undergoing the final ecdysis, and winged gallicolae eventually appeared. At Glen Tress the dehiscence of the galls had become general on 2 September, when mature adults were found.

The comparative paucity of galls of the current season and consequent sparseness of winged gallicolae made it difficult to maintain competent observation of their behaviour in the field. Nevertheless, their migration from Sitka spruce to Douglas fir was verified and oviposition occurred on the latter host (Pl. XXV, fig. 4).

With the gallicolae reared in the laboratory, an experiment was made to determine the readiness with which they would pass to Douglas fir. A young tree, planted in a large pot, was exposed on 2 September in a muslin cage with ripe galls, from which gallicolae were emerging. On attaining their wings, the gallicolae remained quiescent for a few hours before taking flight, when they dispersed to the walls and roof of the cage. After a day's interval a few were observed to have settled on the leaves of the Douglas fir and commenced oviposition 3 days later. Like the sexuparae, the gallicolae secrete a copious amount of waxen wool, investing both their bodies and the egg mass. The eggs began hatching on 14 September.

As there appeared to be no good reason why winged gallicolae might not settle on Sitka spruce equally with Douglas fir, a further experiment was made to test this. On 11 September a young Sitka spruce was placed in the cage but was entirely neglected by the winged gallicolae therein. Specimens of the latter were then transferred to the needles by hand on 15 September. The majority failed to settle and abandoned the needles soon after the transference was made. The few that remained secreted wool. Oviposition was first noted on 18 September, and hatching commenced on 30 September.

It might appear as if the results of this second experiment were suggestive of the possible existence of a "parallel series" of *A. cooleyi* confined to Sitka spruce and comparable to that which occurs on Douglas fir. But further consideration reveals that the results are open to serious criticism. In the first place, the winged gallicolae did not voluntarily select the host as they did the Douglas fir, but were transferred to the Sitka spruce by hand. Secondly, of the 300 odd specimens that were thus transferred, only about a dozen became established and oviposited. Whilst, as was to be expected, the resulting first-stage nymphs (sistentes) resembled in all respects those which hatched from the eggs laid on Douglas fir, it still remains to be seen whether they will continue to thrive and complete their development in the spring. The suitability of the spruce as a host for the progeny of the gallicolae was questioned soon after they hatched, when it was observed that many of them dropped from the needles after making vain attempts to penetrate the leaf tissue with their stylets. There were still a few adherent on 20 December, and their progress is being carefully watched.

Great importance cannot be attached to the fact of oviposition of winged gallicolae on the spruce in the foregoing experiment. In itself oviposition is not an index of host preference, especially where the conditions are widely divergent from those obtaining in Nature. Even the absence of a host tree does not inhibit oviposition. Mature gallicolae that had emerged from galls placed on a white sheet of paper under a glass bell-jar laid eggs not only on the paper but also on the wall of the jar.

Conclusions concerning the distribution of the gall-forming generation cannot be drawn from the few instances of inspection quoted above (p. 590). Nevertheless, the finding of the galls in practically all of the few localities visited suggests that the distribution of the gallicolae and their establishment on the primary host may be fairly wide. The importance of the problem demands the immediate institution of an intensive survey to define the limits of their spread.

(3) *Generation III—colonici*

(*a*) *Categories of sistentes.*

It was not originally planned to devote much attention to the development of generation III (Pl. XXV, fig. 5), the annual cycle of which was reported by Chrystal in 1922 as identical in Britain and America (Text-figs. 1, 2). As the season advanced it became apparent, from such observations as were made, that the cycle in Scotland was not quite so simple as Chrystal (3) had represented (Text-fig. 2) in the south of England. It was, therefore, decided to submit the colonici to closer observation. According to Chrystal there are two component broods in the annual cycle of the colonici, of which one, the sistens, or winter brood I, hibernates as nymphs of the first stage, developing into stem-mothers (fundatrices spuriae) during the following spring. The other, the progrediens, consists of the progeny of the antecedent winter brood I and is the dimorphic summer brood I. One-half of the latter develops into winged migrants, the sexuparae, the other into stem-mothers like those of the winter brood. Finally the progeny of the stem-mothers of the summer brood are said by Chrystal to consist entirely of sistentes, which brings the cycle back to the point where it began the previous year.

Reference to Table I and to Text-fig. 3 will serve to simplify the interpretation of the data, which I obtained during the course of my observations of the colonici at Glen Tress and elsewhere in Scotland, extending from February to December 1935. Comparison of Text-figs. 2 and 3 will show wherein my observations differ from those of Chrystal.

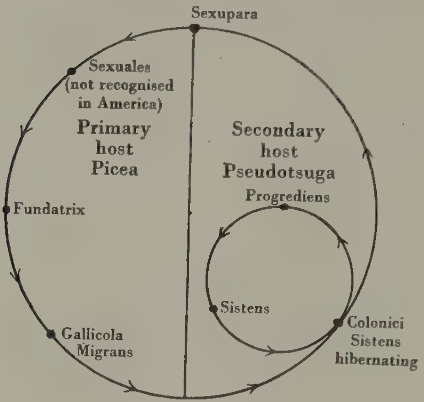

Sexupara
Sexuales (not recognised in America)
Primary host Picea
Secondary host Pseudotsuga
Fundatrix
Progrediens
Sistens
Gallicola Migrans
Colonici Sistens hibernating

Fig. 1

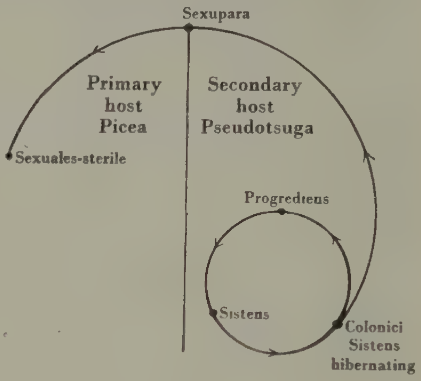

Sexupara
Primary host Picea
Secondary host Pseudotsuga
Sexuales-sterile
Progrediens
Sistens
Colonici Sistens hibernating

Fig. 2

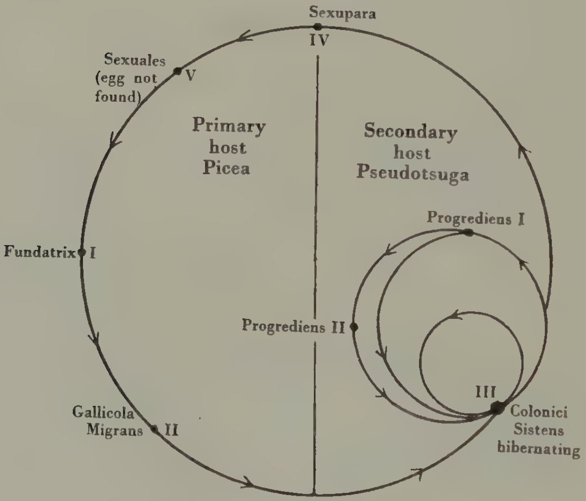

Sexupara
IV
Sexuales (egg not found)
V
Primary host Picea
Secondary host Pseudotsuga
Progrediens I
Fundatrix
I
Progrediens II
III
Gallicola Migrans
II
Colonici Sistens hibernating

Fig. 3

Text-fig. 1. Graphical representation of the life cycle of *Adelges cooleyi* Gillette in America. After Chrystal, *Bull. For. Comm.*, Lond., No. 4, 1922.

Text-fig. 2. Graphical representation of the life cycle of *Adelges cooleyi* Gillette in Britain. After Chrystal, *loc. cit.*

Text-fig. 3. Graphical representation of the life cycle of *Adelges cooleyi* Gillette in Scotland, 1925, as described in the text. Original.

Table I and Text-fig. 3 indicate that the sistentes of winter brood I of the colonici, present on the needles of Douglas fir during any one winter, are a heterogeneous mixture of progeny derived from four different sources as follows:

(1) The whole of the progeny of the gallicolae migrantes which settle on Douglas fir. Sistentes of this category were first observed in September (Table I) and are represented in Text-fig. 3 by the lower arc of the large circle.

(2) Part of the progeny of the stem-mothers of winter brood I of the previous year. The sistentes of this category were first noted in June (Table I). They apparently arose from eggs laid by late-maturing stem-mothers. This part of the cycle is represented by the whole circumference of the inmost of the three small circles (Text-fig. 3), and it will be noted that here a sistens brood gives rise directly to another sistens brood. Progrediens I or summer brood I and the sexuparae are likewise the progeny of the stem-mothers of winter brood I, and are derived from eggs deposited by early-maturing stem-mothers of this brood. This dimorphism is indicated in Text-fig. 3 by the arc of the large circle which proceeds to generation IV (sexupara) and diverges from that of the circle which leads to progrediens I. The origin of the two arcs from a common stem expresses the identity of the first three instars of both summer brood I and sexuparae, the third moult differentiating either a stem-mother of summer brood I or a fourth-instar nymph of the sexuparae with rudimentary wings, which undergoes a further ecdysis before transforming to the winged imago. Both Gillette (4) and Chrystal (3) had previously described this dimorphism.

(3) Part of the progeny of progrediens I or summer brood I. The sistens first-stage nymphs of this category were first noted in July. In Text-fig. 3 they are represented by the free arc of the intermediate one of the three small circles. The remainder of the progeny of summer brood I constitute progrediens II or summer brood II.

(4) The whole of the progeny of progrediens II. The sistentes of this category were first noted in August and are represented by the bottom arc of the outermost of the three small circles.

(*b*) *Annual number of broods.*

In brief, the results of these investigations in Scotland lead to the conclusion that the colonici generation is triple-brooded, consisting of a winter and two consecutive summer broods in contrast to the two broods, winter and summer, found by Chrystal in the south of England

and America. Further, the sistentes in Scotland are composed of the progeny or part progeny of gallicolae, previous sistentes, summer brood I and summer brood II. The contribution of the gallicolae and summer brood I to the population of the sistentes was noted by Chrystal in America, but in the south of England, by reason of the absence of the gallicolae, only the contribution of summer brood I was noted.

My contention that the colonici are triple-brooded recently received unsolicited support in a letter sent by Mr Allan D. Heriot, Entomological Branch, Department of Agriculture, Ottawa, in answer to a request of mine for specimens of the galls of *A. cooleyi* from British Columbia, which duly arrived. Mr Heriot has devoted considerable attention to the Adelgidae of the Interior of British Columbia, and under date of 24 July he wrote: "*A. cooleyi* Gillette migrates from the spruce galls to Douglas fir; the eggs hatch into hibernating first instars, which become foundresses of three generations of apterous forms on the fir, the last generation remaining as a sistens in the first-instar stage all summer and hibernating in this stage. These (sistentes) become foundresses likewise of three generations of apterous forms the following year, except that half the second generation develop into winged forms that fly to spruce."

(4) *Generation IV—sexupara*

(*a*) *Migration to Sitka spruce.*

Generation IV is single-brooded like all the others except the colonici, which has been shown to be triple-brooded. It is greater in numbers than any other generation save the colonici and sexuales, the latter of which is seriously depleted during development (p. 602) by an extensive but unexplained mortality. As with the sexuales and the other winged generation, the gallicolae, there are five instars. The migration of this generation to Sitka spruce from the Douglas fir on which it is bred is readily observable, and its infestation of the primary host may be so intensive (Pl. XXV, fig. 6) as to change temporarily the aspect of the tree. Whilst the early instars of the winged gallicolae are hidden in the closed loculi of the gall, those of the winged sexuparae are exposed on the leaves of the Douglas fir.

By his experiments and observations in the south of England, Chrystal (3) was entirely successful in demonstrating the fact of migration of the sexuparae from the secondary to the primary host and, incidentally, he supplied proof additional to that which he had previously obtained in his Canadian investigations, that Gillette's *Adelges cooleyi* var. *coweni* was merely the colonici stage of *A. cooleyi*, a conclusion which Börner (1) had already reached, based upon the structural affinity of the two first-stage winter nymphs (fundatrix vera and sistens) and their occurrence in the same locality. It only remained to confirm the migration of the sexuparae in Scotland, and this proved a comparatively simple task. My observations differed from those of Chrystal only in regard to dates, since the seasonal appearance of sexuparae and, likewise, the other generations in the south of England, would appear to precede those of the same generations in Scotland by a week or more.

In the laboratory at Edinburgh, fourth-stage nymphs with characteristic wing pads were first observed on an infested Douglas fir on 3 June, but it was not until 10 June that the brownish black-winged adults appeared. This particular Douglas fir was enclosed in a muslin cage with a non-infested Sitka spruce, and after a few days sexuparae were found to have settled on the under surface of the needles of the latter in large numbers. They proceeded to secrete a copious investment of wool and laid ten to twelve brown eggs. As is typical of all generations of the Adelgidae, the eggs were laid in batches, and each egg anchored to the leaf surface by a filamentous stalk arising from its posterior pole. Compared with the gallicolae, each of which lays 100–150 eggs, the reproductive capacity of the sexuparae is about one-tenth. This striking disparity is, however, more than compensated by the superior numbers of the latter, at least in Scotland. The egg mass lies immediately behind the individual which laid it, in the space between the wings, which project well beyond the end of the body and are at the same time inclined to each other over the body. Thus the resting position is identical with that of the ovipositing gallicolae (Pl. XXV, fig. 4).

Oviposition was practically completed by 25 June in the laboratory, and the eggs had almost all hatched by 2 July, at a time when eggs in the field had not yet begun to hatch. The period of incubation in the laboratory varied from 7 to 10 days; in the field it was 10–12 days.

At Glen Tress active migration of the sexuparae from Douglas fir to Sitka spruce was observed on 26 June, when the latter bore a distinctly "frosted" appearance by reason of the large number of individuals, enveloped in white waxen wool, that had become established thereon. It was evident that the migration had been proceeding for at least a week, since many of the sexuparae examined had laid their full quota of eggs (ten to fifteen). At the same time, on the Douglas fir, fourth-stage nymphs were still abundant, and their continued evolution provided a stock from which winged adults were produced, and migration was maintained, although in gradually lessening degree, until the third week of July. In the early stages of the migration the sexuparae settled preferably on the old needles, but later they settled indiscriminately on both old and new needles, and it was not difficult to find individual needles with as many as fifteen sexuparae attached. By the first week of August oviposition had been completed at Glen Tress, and the majority of the sexuparae had died. Their dead bodies, however, remained adherent to the needles for several weeks.

(*b*) *Occurrence on other spruce hosts.*

Sexuparae were observed in localities other than Glen Tress, namely, Melrose, Murthly and Dreghorn. At Melrose, on 26 July, they were found on *Picea alba*, on which, as has been already remarked (p. 590), dead galls of *Adelges cooleyi* had been previously observed. Identification of sexuparae on *Picea nigra* and *P. pungens* var. *glauca* by Laing (5) was also mentioned on p. 590. At Murthly and Dreghorn they were recovered from Sitka spruce. Norway spruce planted with Sitka at Glen Tress was entirely neglected by the sexuparae, and, during and after the migration, the non-infested condition of the former stood in marked contrast to the infested condition of the latter.

(5) *Generation V—sexuales*

(*a*) *Problematical causes of high mortality.*

Little can be said concerning the sexuales except that my observations confirm those of Chrystal (3) concerning the unwonted mortality which results in almost complete annihilation of the whole generation before it attains maturity. At Glen Tress the insects appeared to be decimated at the time of the first moult and for no apparent reason. The first-stage nymphs were peculiarly inactive, and, so far from dispersing, they remained for the most part where they had hatched, in groups of seven to twelve, beneath the shelter of the maternal wool. They succeeded in inserting their mouth-parts into the leaf tissue, but appeared to do little feeding. The abundance of exuviae found adhering to the leaves demonstrated that the first ecdysis took place, but the second and later instars were so reduced as to render the finding of adult males and females a matter of considerable difficulty. As a result only a few specimens of either sex were obtained, and of these the majority were males. Oviposition was not observed and the egg has not been recovered. That it does occur is verified by the existence of the foundresses of generation I, the insignificant numbers of which are explained by the decimation of the antecedent sexuales.

The factors which are responsible for the high mortality of the sexuales still await investigation. It is possible that, as Chrystal has suggested, predaceous mites may be the agents of destruction, but in Scotland I did not find any evidence to support this theory. Further, it seems difficult to accept his suggestion that the preponderance of males is due to their greater activity and, therefore, less liability to attack than the more sluggish females. In any case the total numbers of mature sexuales is so small as to preclude accepting an explanation based on predaceous mites, the attacks of which have not been actually observed.

All the evidence at present points to the operation of an inherent constitutional weakness of the sexuales, which may be accentuated by untoward climatic conditions and maladaptation to the primary host. It will be interesting to learn whether, as time passes, the sexual generation will become more thoroughly established in Britain and result in an increase of the foundresses of generation I and, consequently, of the gallicolae. The occurrence of these two generations in Britain to-day, where they were hitherto unknown, suggests that it has in some degree overcome its inherent weakness.

(*b*) *Occurrence of sexuales in British Columbia.*

In regard to the absence of the sexual generation in America, which Chrystal noted, a further quotation from Mr Heriot's letter in continuation of the one already made (p. 600) shows that this generation is now recognized in British Columbia. Referring to generations IV and V, Mr Heriot proceeds to say: "Many of these winged forms are sexuparae which lay eggs that develop into males or females. In no case have I found both the sexes from the same parent. The female develops a single egg, but the egg has not been found either on the branches, leaves, or beneath the bark scales." Incidentally Mr Heriot's interesting observation that the progeny of individual sexuparae are differentiated as one sex or the other, is one which has not, so far as I am aware, been previously made with regard to *Adelges cooleyi*.

V. SUMMARY

1. *Adelges cooleyi* is a pest of Sitka spruce, the primary host, and Douglas fir, the secondary host, on the latter of which it has been known in Britain since 1913.

2. Hitherto its life cycle in Britain has been regarded as incomplete because of the non-viability of generation I (sexuales) on Sitka spruce and consequent absence of the subsequent generation II (fundatrix vera) and generation III (gallicolae).

3. Both of these generations have been discovered for the first time in Britain and their development followed.

4. The investigation began with the finding of the dead galls of *A. cooleyi* on Sitka spruce at Glen Tress in February 1935. Subsequently, dead galls were also found at Stobs, Melrose, Dreghorn and Murthly. At Melrose they were found on a new secondary host, the white spruce, *Picea alba*. Later the fundatrix vera of generation I was found.

5. The growth of the galls was followed on cut twigs in the laboratory and on growing shoots in the field; galls of the current season were found at Glen Tress, Dreghorn and Murthly.

6. The winged gallicolae of generation II were observed migrating to Douglas fir, where they settled and oviposited. From the eggs there hatched typical hibernating first-instar nymphs (sistentes).

7. Generation III (colonici) was determined to be triple-brooded, and this character has recently been noted in *Adelges cooleyi* in British Columbia.

8. The population of the sistentes on Douglas fir during any one winter was found to be composed of four categories, which have been designated.

9. The migration of generation IV (sexuparae) to Sitka spruce and white spruce was observed at Glen Tress and Melrose respectively. It was also verified in the laboratory.

10. Generation V (sexuales), for some cause or causes yet unknown, was practically exterminated during development. Only a few adults were observed. The solitary egg laid by the female of this generation was not discovered.

11. Further investigation is required concerning:

(*a*) The causes of mortality of the sexuales.

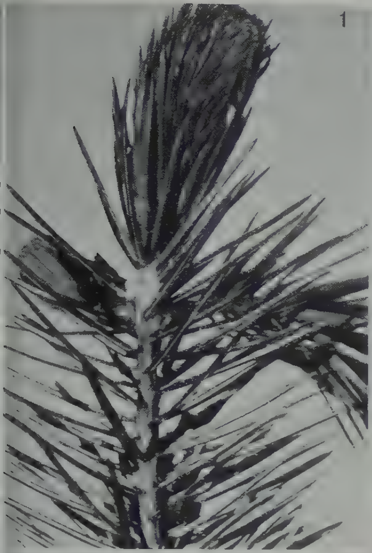
(*b*) The oviposition of the sexual female, and the sexually produced egg.

(*c*) The extent of the distribution and establishment of the spruce generations in Britain.

(*d*) The prospective status of *A. cooleyi* as a pest of Sitka spruce in Britain.

12. With regard to status, it would be unsafe, at the present juncture, to speculate, especially as the investigation has been conducted for only one year. I have, therefore, refrained from discussing possibilities of damage to the spruce host until more evidence is available.

The writer is indebted to Mr R. J. Carrick, B.Sc., Research Student in the Department of Entomology, for the photograph of the winged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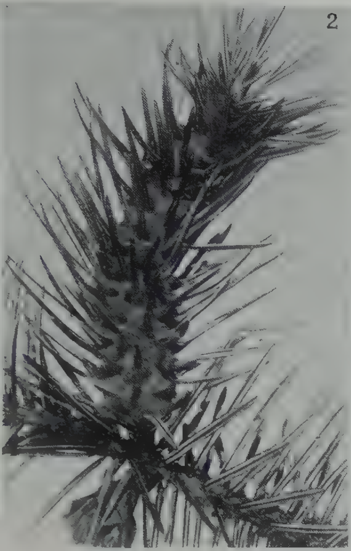
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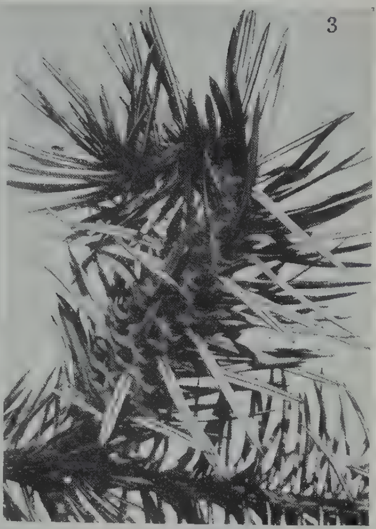
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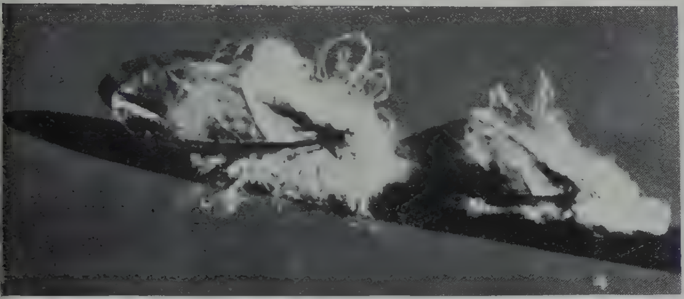
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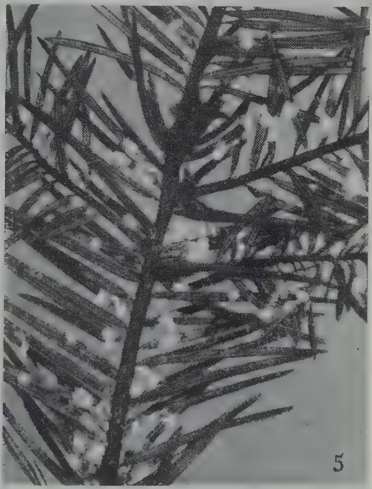
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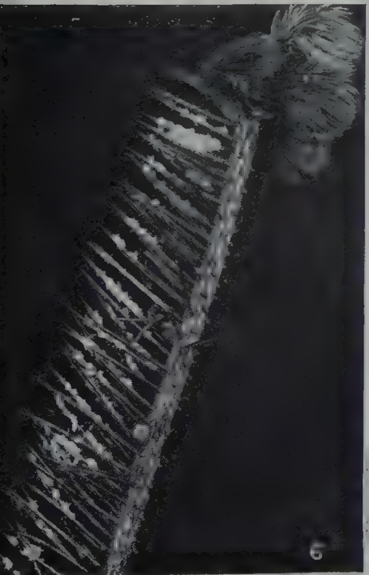
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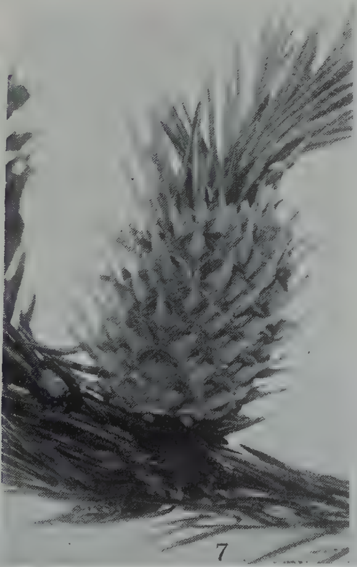
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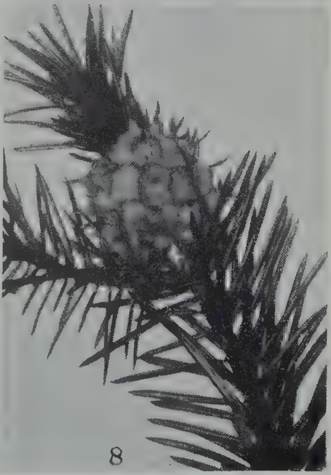
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CAMERON—*Adelges cooleyi* Gillette (Hemiptera, Adelgidae) of the Douglas Fir in Britain (pp. 585–605)

gallicollae reproduced in Pl. XXV, fig. 4. Part of the expenses incurred in conducting the investigation were met by a grant from the Earl of Moray Endowment of the University of Edinburgh, and it is a pleasure to acknowledge this assistance.

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EXPLANATION OF PLATE XXV

Fig. 1. Egg mass of fundatrix or stem-mother (generation I) of *Adelges cooleyi* at base of apical bud of Sitka spruce (*Picea sitchensis*). The egg mass had hatched, and the first-instar gallicolae had settled inside the bud, which was already retarded in axial growth and incipiently galled. Compare the apical with the normally developed right subapical bud or shoot. The left subapical bud was killed by frost. × ½. Original.

Fig. 2. Unopened gall of *Adelges cooleyi* on Sitka spruce. The galled bud is a lateral. The corresponding bud of the opposite side of the shoot (below, in the photograph) had been killed by frost. × ½. Original.

Fig. 3. Open, dead gall of *Adelges cooleyi* on Sitka spruce. × ½. Original.

Fig. 4. Winged gallicolae (generation II) of *Adelges cooleyi* ovipositing on needle of Douglas fir. × 12. Original. (Phot. R. Carrick.)

Fig. 5. *Adelges cooleyi* (generation III) on Douglas fir in late summer. The white woolly masses are the waxen secretions of underlying stem-mothers of summer brood II. The needles are heavily infested with hibernating first-instar nymphs (sistentes) discernible under a magnifying lens. × ½. Original.

Fig. 6. Winged sexuparae of *Adelges cooleyi* on needles of Sitka spruce. The photograph illustrates their abundance. × ½. Original.

Fig. 7. Unopened gall of *Adelges abietis* on Sitka spruce. Compare with Fig. 2. × ½. Original.

Fig. 8. Unopened gall of *Cnaphalodes strobilobius* on Sitka spruce. Compare with Fig. 2. × ½. Original.

(*Received* 23 *December* 1935)

FURTHER EXPERIMENTS ON THE CONTROL OF THE HOP RED SPIDER MITE, *TETRANYCHUS TELARIUS* L.

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In a previous paper (2) experiments were described in which a number of spray fluids were tested for their toxicity to *Tetranychus telarius* on hops. The phytocidal effect of the sprays upon the foliage and inflorescence of hops was also recorded. The results obtained were not deemed conclusive, especially in the case of preparations containing Derris root and those composed of "water-white" petroleum-oil emulsions; the former showed erratic behaviour in their toxicity toward the mites, while the latter, though markedly acaricidal, produced a condition of oedema resulting in severe leaf-fall.

Trials in 1935 were designed to obtain more information upon the toxicity of Derris preparations and the effects of petroleum-oil washes, as well as to investigate the possible acaricidal properties of certain fluids not tested in the previous year.

Lay-out of trial

Thirty-three large pots, each containing a single hop plant, were set out in eleven groups of three pots each. Pots within any one group were separated from one another by about 4 ft. and each group of pots was separated from adjacent groups by at least 6 ft. The spacing was arranged to minimize any chance of possible migration or adventitious carriage of mites from one pot to another.

Each plant produced at least one strong bine and in some instances two. Individual bines were trained separately on poles and most of them eventually reached a height of 6–8 ft.

The plants were all kept with the minimum amount of water, a condition which appears to favour the survival and rapid multiplication of the mites, but they were all equally subjected to any rain which fell, since the pots were grouped on a large cinder bed in the open.

A group of three plants was taken as the unit for any one treatment. Ten spray fluids were applied and one group of pots retained as untreated controls. The treatments were as follows:

(1) *Lime sulphur* at a concentration of 1 gallon of lime sulphur in 79 gallons of water.

(2) *Petroleum-oil emulsion*, the wash containing 2 per cent. oil and 1 per cent. whale oil-potash soap.

(3) *Bordeaux mixture-cotton-seed oil emulsion*, the wash containing 6 pints edible cotton-seed oil per 100 gallons of Bordeaux mixture (10 : 15 : 100).

(4) *Petroleum-oil emulsion*, the wash containing 1 per cent. oil and ½ per cent. whale oil-potash soap.

(5) *Derris extract* "*A*", the wash being made up to contain 0·005 per cent. rotenone.

(6) *Whale oil-potash soap*, at 1 per cent. concentration.

(7) *Whale oil-potash soap*, at ½ per cent. concentration.

(8) *Derris extract* "*B*", the wash being made up to contain 0·002 per cent. rotenone.

(9) *Bordeaux mixture* (10 : 15 : 100) + *a Pyrethrum extract*, the wash containing 0·0025 per cent. of pyrethrins 1 and 2 combined.

(10) *Pyrethrum extract*, the wash containing 0·0025 per cent. of pyrethrins 1 and 2 combined.

(11) Untreated controls.

APPLICATION OF THE WASHES

By mid-July a considerable population of *T. telarius* had established itself upon the plants, the foliage was beginning to show characteristic signs of attack, and the mites had produced quantities of silk web on the undersides of the leaves. As far as could be judged visually, the infestation was more or less uniform over the whole series, and at any rate all the lower leaves of the plants carried a large mite population. Two applications of each wash were made, on 23–24 July and 1–2 August respectively. A pneumatic knapsack machine was used for the purpose. All washes were applied at the same pressure and in the same manner, the liquid being directed upward at the undersides of the leaves. Sufficient wash was used to ensure thorough wetting of the foliage and approximately equal amounts of each were applied, though there was necessarily some variation owing to differences in the size of the plants.

ESTIMATION OF RESULTS

Between 1–30 August six estimations were made on the plants to assess the effects of the washes. The majority of leaves on all plants were examined on each occasion, but particular attention was paid to those on the lower 5 ft. of the bine. Very little variation in the intensity of attack occurred during the period when observations were made, and the toxicity of the washes, as noted on the day after the second application, continued to give a true indication of the degree of infestation throughout August. Plants which were cleared of mites did not again become appreciably infested, but, where no control of the mites was obtained the population continued to develop and the plants were very badly attacked. A summary of the observations made throughout August is given below.

Treatment 1. Very few mites, plants almost completely free.

Treatment 2. Very few mites. Leaf-fall commenced about 6 days after first application of the wash.

Treatment 3. Mites numerous, no appreciable reduction in numbers.

Treatment 4. Very few mites. Leaf-fall as (2).

Treatment 5. Mites plentiful.

Treatment 6. Mites plentiful.

Treatment 7. Mites plentiful.

Treatment 8. Mites plentiful.

Treatment 9. Mites plentiful.

Treatment 10. Mites plentiful.

Treatment 11. Very heavy infestation, plants swarming with mites.

DISCUSSION OF RESULTS

In confirmation of the results obtained in 1934, both in small-scale and field trials, lime sulphur showed marked toxicity to the mites at a concentration of 1/80. There was no indication of any phytocidal effect following its use.

Petroleum oil was quite equal in toxicity to lime sulphur, but phytocidal effects were apparent at a concentration as low as 1 per cent. The specification of the oil used, however, places it in the category of those likely to cause chronic injury to plants, by reason of its high boiling range and viscosity, for, according to the evidence assembled by Martin(4), oils of relatively low viscosity are less likely to produce metabolic disturbances in plant foliage than those of higher viscosity, assuming the oils are of such a character that the injury is not attributable to chemical properties. The specification of the oil used is given below.

Boiling range: 10 per cent. distilled up to 378° C.
30 per cent. distilled up to 391° C.
50 per cent. distilled up to 400° C.

Viscosity: Redwood 1 at 70° F., 806″.

Unsulphonated residue: 100.

Specific gravity at 60° F.: 0·885.

Its high boiling range and viscosity indicate that the oil would form a very stable film upon foliage and, until there is more evidence regarding the effect of petroleum oils of lower viscosity upon hops, they must be regarded as potentially unsuitable for use.

The soap employed for the purpose of preparing the oil emulsions was shown to produce no phytocidal effects at the concentrations employed, neither did it show any toxicity toward the mites.

Cotton-seed oil has been shown by Martin (3) to have some insecticidal value against aphides, and Austin *et al.* (1) have employed it in conjunction with Bordeaux mixture for the preparation of insecticide-fungicide combination washes on fruit trees. It could be used in a similar manner by hop growers, and it was thought desirable to discover whether the cotton-seed oil possessed any toxicity toward "red spider". At the concentration employed it proved to be quite non-toxic.

Both the Derris extracts were without appreciable effect, and though they brought about a very slight reduction in the number of mites, as far as could be judged, the mortality was so small that it could not be assessed by the method employed and was not comparable with that caused by lime sulphur and petroleum oil. The washes containing pyrethrum extract also proved quite ineffective.

Control measures in commercial practice

In applying lime sulphur for the control of "red spider" in the field, two main difficulties are encountered: first, the method of application of washes by means of the usual hop-washing machine may not give a sufficiently good cover on the undersides of the leaves, and second, there may be injurious effects resulting from the interaction of lime sulphur and Bordeaux mixture upon the foliage. It is unavoidable, in commercial practice, that these two substances shall be applied with a very small time interval between them, for regular applications of Bordeaux mixture are normally made in July for the control of Hop Downy Mildew, at a time when it is imperative also to take measures against "red spider".

In a hop garden near Canterbury, which was very badly attacked by *T. telarius* in 1933 and 1934, Mr W. J. Rickards tried out a suggested spray programme over several acres on the variety "Fuggles". The observations recorded below were obtained from this hop garden.

Winter treatment

In order to reduce the initial infestation of hops in the spring by female mites emerging from hibernation, the poles were treated with a tar oil winter wash at 6 per cent. concentration. Knapsack machines were used for the purpose and the wash was directed into the main cracks of the poles, the nozzle being held very close to them and the liquid forced in as thoroughly as possible. The poles were only washed to the height which a man could easily reach when standing on the ground and holding the spray lance out to its maximum distance. Thus no wash was applied to the poles at a greater height than about 8–9 ft. and the tops were left untouched. It has previously been shown by Jary (2) that the number of hibernating mites is usually small in the top few feet of the poles, and that the great majority are assembled in the part from ground-level up to a height of about 5 ft.

In the spring of 1935, following the treatment of poles with a tar-oil wash, the original infestation of the hops was small. It should be noted, however, that abnormal weather conditions at this period probably affected the mite population to some extent. By July, when drought conditions prevailed, small colonies had become established and might, if weather conditions remained favourable for them, have become widespread in time to cause serious injury. It was therefore thought advisable to attempt to control the attack before the mites became more numerous.

An application of Bordeaux mixture had been made on 4 July at the rate of about 400 gallons per acre, and it was still very much in evidence on the leaves on 18 July, when lime sulphur at 1/80 was applied. The lime sulphur was a proprietary brand without wetter incorporated, and a wetter of the sulphonated lorol type was added to it. This substance is known to be harmless to hop foliage and to have no toxic action upon the species of mite concerned (Jary (2)). About 400 gallons of lime sulphur per acre were applied and the wash was concentrated on the bine up to the height of the bar string, about 9 ft. from the ground, the nozzles on the hop washer being rearranged for this purpose and no attempt was made to reach the tips of the bines, where no mites were present. The lime sulphur wash was thus concentrated on the lower half or two-thirds of the foliage only and this received, therefore, a very heavy application. On 22 July no injury was apparent on the hops, and another application of Bordeaux mixture, similar in amount to the first, was made. The interaction of the two spray fluids produced dark deposits all over the leaves and the foliage presented a very dirty appearance, but no injury was caused and there was no abnormal leaf-fall.

On 26 July a very thorough examination for the presence of *T. telarius* was made all over the acreage concerned, but only a very few isolated mites could be found, together with a small number of eggs. For all practical purposes the attack was suppressed and it was not found necessary to repeat the lime sulphur application.

SUMMARY

1. Small-scale experiments with a number of spray fluids gave the following results against *Tetranychus telarius* on hops:

(*a*) Lime sulphur at a concentration of 1/80 proved very toxic to the mites, but a second application had to be made to deal with those which hatched subsequently from eggs.

(*b*) Emulsions of a "water white" petroleum oil at 2 and 1 per cent. concentrations were very toxic to the mites but caused marked oedema.

(*c*) Whale oil-potash soap at 1 and ½ per cent. concentration; cottonseed oil emulsion, 6 pints of oil per 100 gallons of wash; Derris extracts of 0·005 per cent. rotenone and 0·002 per cent. rotenone content, and a pyrethrum extract of 0·0025 per cent. content of pyrethrins 1 and 2, were all without appreciable toxic effect upon the mites.

2. Lime sulphur at 1/80, to which a wetter of the sulphonated lorol type was added, proved successful in the field for the control of *T. telarius* on hops. No injury to the plants resulted from the use of Bordeaux mixture on the same plants.

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THE BIOLOGY OF THE CHRYSANTHEMUM LEAF-MINER, *PHYTOMYZA ATRICORNIS* MG. (DIPTERA: AGROMYZIDAE)

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(With Plates XXVI and XXVII and 8 Text-figures)

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INTRODUCTION

GLASSHOUSE horticulture has now reached the standing of an important industry in many parts of the British Isles. Glasshouse areas are to be found in Lancashire, Cheshire, north and south Wales, the Lea Valley and other districts. Most large towns have such areas on the outskirts of the industrial centres, and amongst the plants raised for ornamental purposes chrysanthemums and other Compositae are high in the list.

In many localities chrysanthemums and other Compositae are subject to attack by the chrysanthemum leaf-miner. In some years these attacks are so severe that the crop is seriously affected. The year 1934–5 proved to be one during which chrysanthemums were badly damaged by this insect all over the country (12).

Although of considerable economic importance the chrysanthemum leaf-miner has not been given the attention it demands in this country, and at the suggestion of Dr H. W. Miles the species as it occurs in the Lancashire area has been studied in detail.

SYNONYMY

In 1838 (8) Meigen first gave the name now adopted for this insect, although Macquart in 1835 (7) had described the same fly as *Phytomyza geniculata*. *P. geniculata* would therefore appear to have priority, and this name was generally used for some time. However, Brullé in 1832 (1) had described another agromyzid as *P. geniculata*, and since *P. geniculata* Macquart and *P. geniculata* Brullé are not synonymous, the former name lapses and the insect under discussion is now generally known as *P. atricornis* Mg. The synonymy of the species is given below:

(*P. geniculata* Macquart, 1835),
P. atricornis Meigen, 1838,
P. horticola Goureau, 1851,
P. tropaeoli Dufour, 1857,
P. linariae Kaltenbach, 1857,
P. pisi Kaltenbach, 1857.

These various names were probably given as the result of assuming that leaf-mining insects show complete specificity in the choice of host plants.

In America chrysanthemums, according to Smulyan (14), are attacked by *P. chrysanthemi* Kowarz. Frost (3), after examining European specimens of *P. atricornis* Mg., states that *P. chrysanthemi* Kowarz is a distinct species. Hendel (4), however, would place *P. chrysanthemi* Kowarz in synonymy with *P. atricornis* Mg.

DISTRIBUTION

The insect has been recorded from many parts of the world and is common and widespread throughout Europe. A study of the Monthly Summaries of the Plant Pathological Laboratory of the Ministry of Agriculture indicates that *P. atricornis* Mg. is present in all regions of the British Isles where composites are cultivated and is particularly troublesome where these plants are grown under glass. Infestation has been especially severe in north Wales, and widespread attacks have been noted in Cheshire, Yorkshire, Surrey, and Devon.

FOOD PLANTS

In addition to cultivated chrysanthemums, according to Seguy (13) *P. atricornis* Mg. has been bred from no less than twenty-three species of composites, including *Cineraria stellata* L., *Calendula officinalis* L., *Helianthus annuus* L.; seven species of crucifers; four species of leguminous plants; three species of labiates, and various plants among the Lilliaceae, Linaceae, Malvaceae, Papaveraceae, Polemoniaceae, Resedaceae, Scrophulariaceae, Solanaceae, and Valerianaceae. In all, no less than seventy-two plants are said to be mined by the larvae of *Phytomyza atricornis* Mg.

INJURY TO THE PLANT

P. atricornis Mg., unlike the chrysanthemum midge (*Diarthronomyia hypogea* F. Lw.) produces larvae which are true miners and not gall makers. The larvae eat through the mesophyll of the leaf, often removing almost the whole of the assimilating tissue. The mine is linear and tortuous and is usually confined to the upper surface of the leaf so that the injury is visible from the abaxial surface of the leaf lamina. Numerous larvae may be found in one leaf, and their mines cross and recross, thus in the final stages giving the effect of a blotch mine.

In addition to the unsightly effect produced by the larvae, the adult female fly is also responsible for a certain amount of disfiguration of the foliage. Of the numerous incisions made by the ovipositor, only a relatively small number are provided with eggs—the others being used as feeding points. On cuttings during the early part of the season these holes remain as small bleached areas visible from the upper surface of the leaf. When the cuttings have made some 3 or 4 in. of growth, and also during the flowering period, the feeding holes are marked by small wart-like growths. These are composed of mesophyll cells which are abnormally enlarged and the growth is hard (Pl. XXVI, fig. 2 and Pl. XXVII, fig. 5).

On chrysanthemums grown out of doors, the injury is seen during the summer and autumn periods. On plants under glass the flies multiply quickly in the periods of rapid growth of the host plant, viz. spring and autumn. They are present throughout the year in glasshouses where Compositae are grown.

In spring, cuttings are liable to be badly attacked and every leaf may be mined, the infestation being much more severe than during the flowering season. Table I shows the relative density of attack on the variety "Edward Page" in April and December.

Table I

Variety "Edward Page"

April		December	
Av. size of leaf in.	No. of puparia per leaf	Av. size of leaf in.	No. of puparia per leaf
1·1	11·2	4·25	7·6

Severely attacked cuttings make very slow growth and may be destroyed in some instances. Generally, however, the plants do not die but make new foliage when they are taken outside to the frames. This may be accounted for by the fact that flies feeding on outdoor host plants emerge later in the year and the cuttings are thus given time to recover from the attack.

Even during the breeding season of the fly the plants only occasionally seem to be severely attacked out of doors, e.g. the season 1934–5. This may be owing to the fact that there is a variety of alternative host plants present, and also that the flies are no longer concentrated in a relatively small area.

NOTES ON THE SEVERITY OF THE ATTACK ON CERTAIN VARIETIES

The relative degree of infestation of several varieties from two heavily infested nurseries was ascertained by measuring the size of leaf and counting the number of living larvae or puparia. Table II gives the results of the examination of six varieties during the flowering period and two varieties in spring. The greatest number of puparia found in any one leaf was fifteen (variety "Edward Page" in November). The figures suggest that where the fly is present it attacks all varieties, the number of puparia being limited by the size of leaf.

Table II

Variety	Av. size of leaf in.	Av. number of puparia per leaf
Jean Pattison	4·0	6·5
Edward Page	4·25	7·6
	1·10*	11·2*
Birmingham	5·5	8·4
Favourite	4·0	3·5
Mary Wallace	2·2	2·8
Sandown	1·3*	5·6*

* Denotes counts made from cuttings.

During the latter part of the summer—in August and September—chrysanthemum plants in the field do not appear to be attacked by *P. atricornis* Mg. Plants which are well advanced towards the flowering period show no indication of fresh oviposition holes and mines.

At this time of the year, as during the summer generally, composite weeds such as *Sonchus oleraceus* L., *Senecio vulgaris* L. and *S. jacobaea* L. are subject to dense infestation, while herbaceous plants such as *Chrysanthemum maximum* L., *C. segetum* L., *Achillea millefolium* L., *A. filipendulina* Lam. and *Artemisia absinthium* L. are severely attacked.

It is possible that during the late summer months the flies migrate to wild host plants in preference to cultivated composites. This point, however, requires further study before a definite statement can be made. Infestation of the plants commences again when they are taken into the glasshouse for the flowering period.

THE LIFE HISTORY OF *PHYTOMYZA ATRICORNIS* MG.

Mating

Extended counts of adult flies at several times during the 1935 season gave the following sex ratio:

Males : Females :: 1·1 : 1.

In no case did one sex greatly outnumber the other, though the male flies tended to emerge before the females.

Mating takes place several hours after emergence and may occur as soon as the flies have obtained their normal appearance. The flies do not appear to be influenced to any great extent by conditions of light and moisture, and were observed to mate in daylight and artificial light and in atmospheres whose relative humidities varied greatly.

The male mounts on the abdomen of the female, separating the wings and grasping the thorax near the wing bases with the anterior pair of legs. The second pair of legs are placed round the middle of the abdomen and the abdomen of the male curves downwards and forwards to meet the tip of the abdomen of the female.

Often two males will attempt to mate with a single female and occasionally males confined by themselves will attempt to mate with each other. In this case the same position is taken up as in actual copulation. The female often remains motionless during copulation but may walk slowly over the leaf. Occasionally vigorous attempts are made to shake off the male, and in this way connexion is generally broken. Sometimes the male himself breaks the connexion by releasing the female and withdrawing the genitalia after facing in the opposite direction to the female. The same female may be mated more than once with the same or different males.

The duration of the copulation period varies greatly from about half a minute to many minutes. One pair remained connected for 30 min. and another for 2 hours. Smulyan (14) states that the average period for *P. chrysanthemi* Kowarz was 30 min. to 1 hour.

After mating the male retracts its genitalia immediately and may fly away at once. The female remains in the same place for a few minutes during which time the ovipositor is partially protruded and retracted while the tip of the abdomen is stroked with the hind legs. As soon as this operation is finished the female may commence to insert the ovipositor into the leaf, either for the purpose of feeding or egg laying.

Feeding

Females were observed to insert the ovipositor into leaf tissue immediately they were released from the breeding tubes, whether fertilized or not. The process is as follows:

The female selects a spot on the leaf surface by testing with the ovipositor. The tip of the abdomen is then bent slightly forwards under the body so as to make an angle of approximately 90° with the leaf surface. The ovipositor is then inserted into the leaf and travels backwards through the tissue just under the epidermis. The terminal segments of the ovipositor are then withdrawn and pushed out alternately, being directed to both sides of the plane of the first insertion. At the same time the abdomen is rotated, the ovipositor sheath being used as a centre. In this way, on complete withdrawal of the ovipositor, a cavity remains which has the shape of a truncated cone, the base of the cone being in the tissue.

The female fly then moves backwards over the incision and immediately applies the proboscis to the sap that exudes from the perforation. Feeding punctures occur most commonly on the upper surface, although they may also be found on the lower surface.

The males are not capable of piercing the leaf tissue but may feed from incisions made by the female flies.

Punctures made for the purpose of feeding are far more numerous than those made for the purpose of egg laying. Some leaves may be greatly scarred with feeding punctures while others have only a few incisions containing eggs. Table III shows the relative number of ovipositor punctures and of larvae in different leaves of the variety "Mary Wallace".

Table III

No. of ovipositor holes	No. of larvae
55	1
95	1
51	3
44	0
9	2
22	0
23	2
37	3
32	3
47	1
100	0

Duration of egg-laying period

To ascertain the duration of the egg-laying period single fertilized females and single pairs of females and males were confined in tubes with leaves. The leaves were removed each day and replaced by fresh ones, all ovipositor punctures being examined for eggs. Some parallel experiments were made, using the leaves of *Cineraria stellata* L. which is also attacked by the fly; in this plant the eggs could easily be seen through the epidermis of the leaves.

One female lived 13 days and oviposited on 12 days. Another lived 9 days and oviposited on 8 days. The majority of the eggs were deposited between the first and third days after the first fertilization. In one instance seventy-two eggs were laid and in another fifty-one eggs.

The maximum number of eggs laid by one female during 24 hours was seventeen. Dissection of fertilized females showed that the number of mature eggs present in both ovaries varied from forty-five—

immediately after mating—to thirty-five after being on the plant for several days. It would appear that all the mature eggs are not laid at one time.

Length of adult life

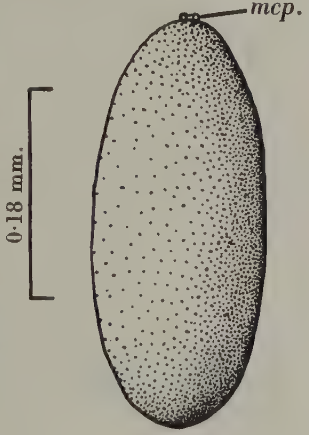
Adult females generally live much longer than males. This is primarily due to the use made by the females of the ovipositor for making punctures for feeding, while the males are unable to pierce the leaf tissue to obtain food.

Female flies kept on leaves lived from 9 to 21 days under laboratory conditions. Males kept with females on leaves lived from 4 to 10 days. As already mentioned, it is possible that in applying the proboscis to the leaf surface male flies imbibe sap from ovipositing punctures made by the female. When males were kept separately they generally died after 3 days. Females died after 3–3½ days if not allowed access to leaves.

The egg (Pl. XXVII, fig. 4)

The egg is ovo-cylindrical with a small but well-defined micropyle at one end and measures 0·36 × 0·15 mm. (Text-fig. 1). The chorion is devoid of reticulation, being smooth and glistening, and the egg is translucent white in colour.

After 24 hours at 18° C. localization of the yolk commences and the egg becomes more translucent in certain parts. After 3 days the embryo is well advanced; segmentation is distinct and the rudiments of the mouth-parts are also apparent. At the end of 3½ days the embryonic period is almost complete and the mouth-parts are quite dark and heavily chitinized. The larva is very restless and moves continually, the movement sometimes resulting in the larva reversing its position in the chorion.

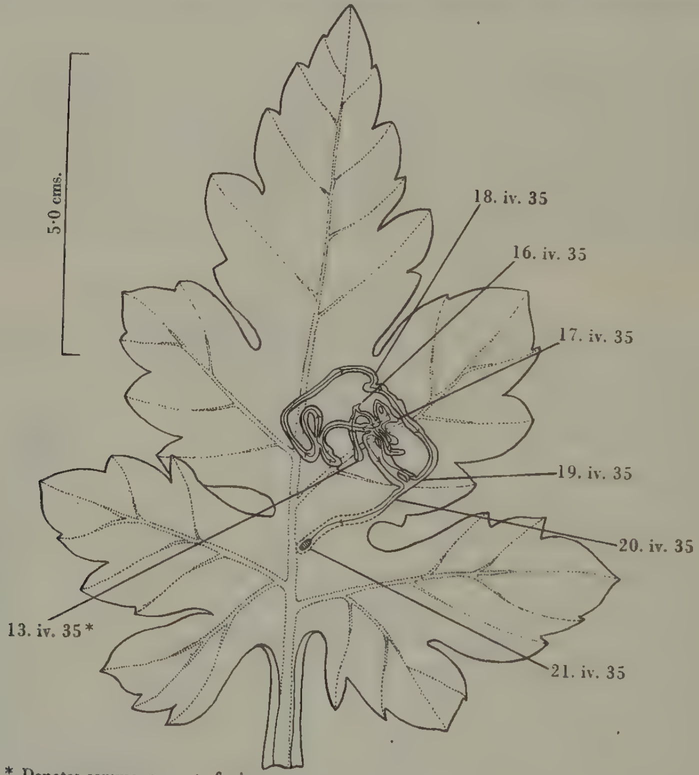


Text-fig. 1. Egg of *Phytomyza atricornis* Mg.
mcp. micropyle.

The eggs were incubated in the laboratory at an average temperature of 18·0° C. Of fifty-one eggs examined for the period of incubation, seventeen hatched after 3½ days and thirty-four after 4 days. Smulyan (14) states that eggs of *Phytomyza chrysanthemi* Kowarz hatched on the average after 5 days, the average temperature being 16·3° C. For *P. atricornis* Mg. the average incubation period would appear to be about 4 days at 18° C.

Emergence, feeding of the larva and construction of the mine

The larva emerges from the egg by rupture of the chorion, but it is not possible to see whether the emergence slit is regular in form for each egg. The larva commences to feed immediately after hatching and usually remains for a varying period in the aerating tissue of the leaf, but may eat immediately right through the mesophyll to the palisade tissue. The mouth hooks of the larva are in the form of two-toothed rakes, which oscillate about a fulcrum situated at the point of articulation with the hypostomal sclerite.



* Denotes commencement of mine

Text-fig. 2. Diagram showing the development of a single mine of *Phytomyza atricornis* Mg.

In feeding the larva lies on the left side, the cephalon and anterior part of the trunk being bent to the left. The mouth hooks oscillate back and forth and remove a swathe of palisade cells. The cephalon now points to the right. The larva moves forwards slightly and commences to remove another swathe of tissue, beginning the cut on the left side as before.

The oviposition hole is thus the commencement of the mine, and generally the mines begin near the leaf margin (rarely more than ½ in. away) and may continue to follow the outline of the leaf for some distance before turning towards the midrib.

The mines are linear and tortuous, and since they are formed by removal of the palisade tissue the upper epidermis is left white and bleached and the mines are therefore very conspicuous from the upper surface of the leaf.

The frass is deposited on the lower surface of the mine as a line of small granules, which are often smeared so that the mine has a brown colour. Where more than one egg is laid in the same leaf anastomosis of the resultant mines is very common and, as already mentioned, the leaf eventually assumes an appearance which might be caused by a blotch miner. It is possible, however, to see the individual tracks, the larvae being primarily solitary feeders.

The third-instar larva leaves the palisade tissue some time before it is mature and makes its way into the aerating tissue, where it eats out a chamber and forms a puparium under the lower epidermis and usually near the midrib.

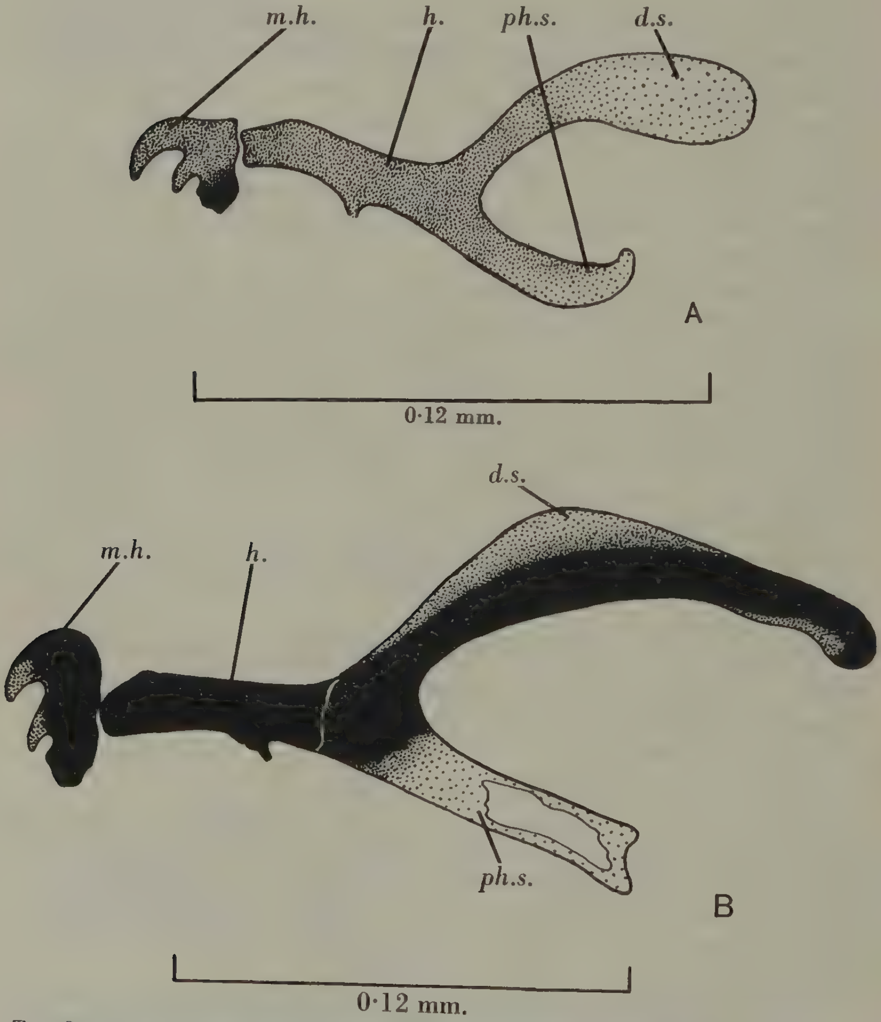
The growth of a single mine from eclosion of the larva to the formation of the puparium is shown in Text-fig. 2, and indications of the daily increments in length are also given.

THE FIRST-INSTAR LARVA

On hatching, the first-instar larva is very minute, measuring 0·44 × 0·15 mm. and is not much larger than the egg. It is smoothly cylindrical and tapers slightly towards the anterior end. The trunk is translucent, the fat bodies and the alimentary canal being visible through the cuticle. When the cephalo-pharyngeal skeleton is retracted, the segmentation of the larva becomes quite marked by contraction of the anterior end of the body.

The head bears paired antennae and maxillary palps in addition to sense organs in the form of minute circular pits. There is no "toothed plate" such as is described by Keilin (6) and de Meijere (9) in *Calliphora erythrocephala* Mg., and Steel (15) in *Oscinella frit* L.

The cephalo-pharyngeal skeleton (Fig. 3 A) consists of paired mouth hooks, articulating with a single hypostomal sclerite which, passing backwards, bifurcates on each side into the dorsal sclerite and pharyngeal sclerite. The hypostomal and pharyngeal sclerites are not separate. The mouth hooks have each two teeth, the apical one being the largest. In this instar the mouth hooks are not as heavily chitinized as the hypostomal sclerite. Accessory sclerites such as are described by Steel(15) in *Oscinella frit* L. are absent, and there is also no connexion between the dorsal sclerites on each side.



Text-fig. 3. Cephalo-pharyngeal skeleton of instars of larva of *Phytomyza atricornis* Mg. A, first-instar larva; B, second-instar larva; C, third-instar larva—lateral view. *m.h.* mouth hook; *h.* hypostomal sclerite; *d.s.* dorsal sclerite; *ph.s.* pharyngeal sclerite.

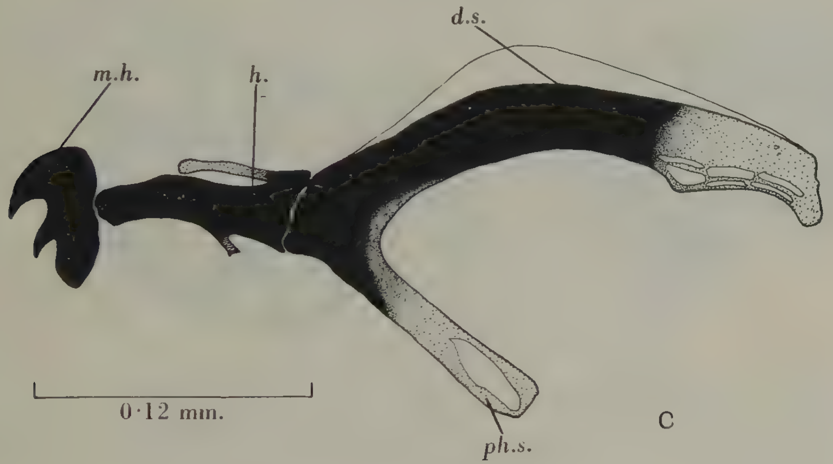
There are eleven body segments, three of which are thoracic. The segmental grooves are not well defined unless the larva is in a contracted state. Each intersegmental groove, with the exception of the first two, is marked by rows of minute blunt denticles arranged in short series instead of continuous lines.

The anus is a longitudinal slit on the last abdominal segment between and ventral to the posterior spiracles.

The first-instar larva is metapneustic, the anterior spiracles being absent. The posterior spiracles are small pores situated on slight prominences on the eighth abdominal segment; they lead into separate stigmatic chambers whose walls are devoid of taenidia. The main tracheal trunks run dorsally in the body and have dorsal commissures in each segment except the prothoracic. The mesothoracic and the eighth abdominal commissures are much stouter than the rest. Lateral tracheal tubes given off from the main trunks are connected by longitudinal commissures, thus forming a secondary lateral trunk on each side.

THE SECOND-INSTAR LARVA

The second-instar larva varies in length from 1·40 to 2·1 mm. and in width from 0·55 to 0·63 mm. The larva is widest in the metathoracic region and tapers off gradually to the posterior end. The cephalic sense organs are similar to those of the first and third instars. The second-instar larva exhibits a lightly chitinized plate slightly ventral to the aperture through which the mouth hooks are exserted; it extends on either side of the mouth hooks, but its margins are indefinite. This plate bears sensory pits and may be homologous with the labium described by Thomas in *Opomyza germinationis* L. (17) and in *O. florum* Fabr. (16).



Text-fig. 3 C.

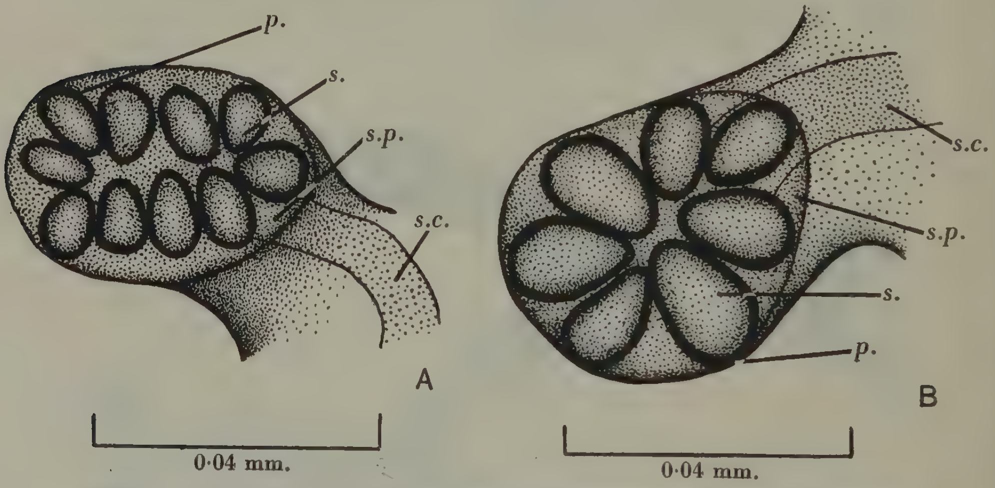
The *mouth-parts* have increased greatly in size and show marked differences from those of the first instar. The mouth hooks are longer, but the teeth remain about the same size as those of the first instar. The hypostomal sclerite is now separate from the pharyngeal sclerite on each side (Fig. 3 B).

Above the cephalic sense organs is a patch of denticles which are much larger and more pointed than those of the segmental grooves. There are similar patches on either side of and just ventral to the mouth opening. These denticles are directed ventrally and must have a scraping action which aids the mouth hooks in the removal of the leaf tissue (Text-fig. 5).

The intersegmental denticles are more numerous than those of the first instar, being especially well marked on the ventral and ventrolateral regions of the body wall. They are less numerous on the dorsal surface.

The second-stage larva is amphipneustic, the anterior spiracles being open to the exterior. The general plan of the tracheal system follows that of the first- and third-instar larvae. The main difference from the first-instar larva is that the dorsal trunks open into the anterior spiracles instead of ending anteriorly in tracheoles. The cephalic and pharyngeal regions of the larva are supplied by tracheae which arise from the dorsal trunks just before their insertion into the anterior spiracular chambers.

The *anterior spiracles* are set dorsally just in front of the posterior margin of the prothoracic segment. They are very close together, and each is situated on the apex of a short stalk so that the spiracle stands away from the body wall. From above, the spiracle is roughly oval in outline and has from nine to ten pores arranged in two irregular rows. These pores are set on the tips of short digitate processes and each pore has a thickened chitinous ring of peritreme. The pores open into the stigmatic chamber, whose walls have a granular appearance and are devoid of taenidia (Text-fig. 4 A).



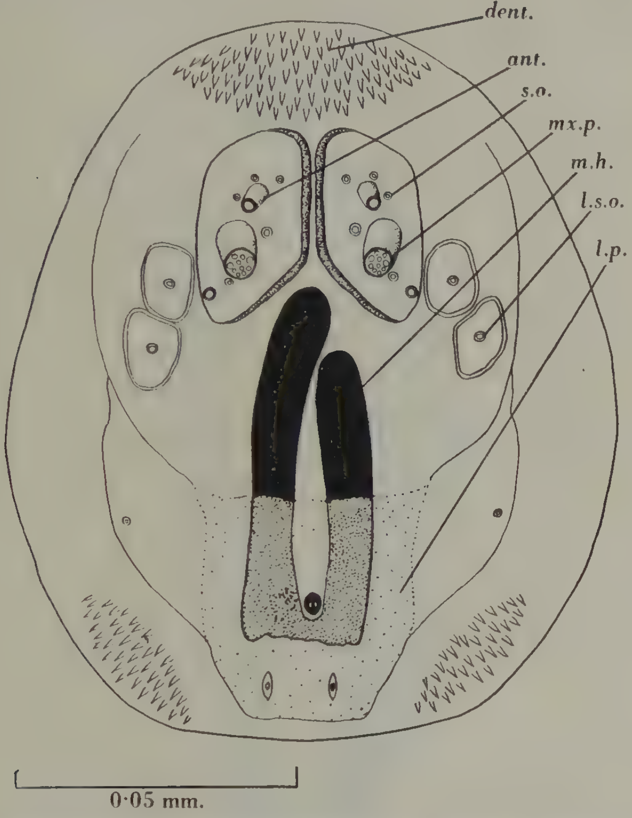
Text-fig. 4. Spiracles of the second-instar larva of *Phytomyza atricornis* Mg. A, anterior spiracle; B, posterior spiracle. *p.* peritreme; *s.* spiracular pore; *s.p.* spiracular plate; *s.c.* spiracular chamber.

The *posterior spiracles* are also set on short stalks and project outwards and upwards. There are only seven pores set on short digitate processes (Text-fig. 4 B). Branched hairs such as are found in *Oscinella frit* L. and *Opomyza* sp. are absent.

The third-instar larva

In this stadium the larva measures from 3·2 to 3·5 mm. long and from 1·5 to 2·0 mm. wide. The trunk is translucent white in colour, the alimentary canal being visible through the body wall in some places. The fat bodies are prominent, and numerous calco-sphaerites are present. These vary in shape but always exhibit a concentric structure. Their calcareous nature is shown by their slow solution in acid alcohol with evolution of gas.

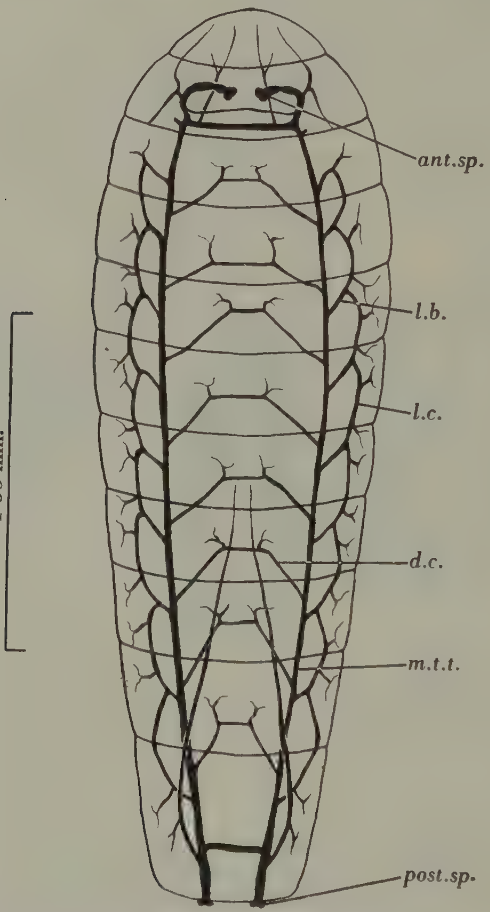
The *head* is small and may be retracted within the prothoracic segment by muscular action. The sense organs are borne on the anteroventral face on what may be termed antenno-maxillary complexes (Text-fig. 5).



Text-fig. 5. Ventral view of head of third-instar larva of *Phytomyza atricornis* Mg. showing antenno-maxillary complex. *dent.* denticle; *ant.* antenna; *s.o.* sense organ; *mx.p.* maxillary palp; *m.h.* mouth hook; *l.s.o.* lateral sense organ; *l.p.* labial plate.

The anteroventral surface of the head is divided into two plates by a longitudinal groove having thickened walls which form the inner margin of each plate. Each plate bears just above the middle line a cylindrical antenna. Directly below the antenna, on each side, is a cylindrical maxillary palp which is larger and bears ten sensory papillae terminally. Three of these papillae are much larger than the rest. In addition the plates each bear six sensory pits of various diameters (Text-fig. 5). There are two lateral sensory pits arranged along the outer margin of each antenno-maxillary plate. These differ from those around the maxillae and antennae in that they are surrounded by a lightly chitinized ring some distance from the sensory pit.

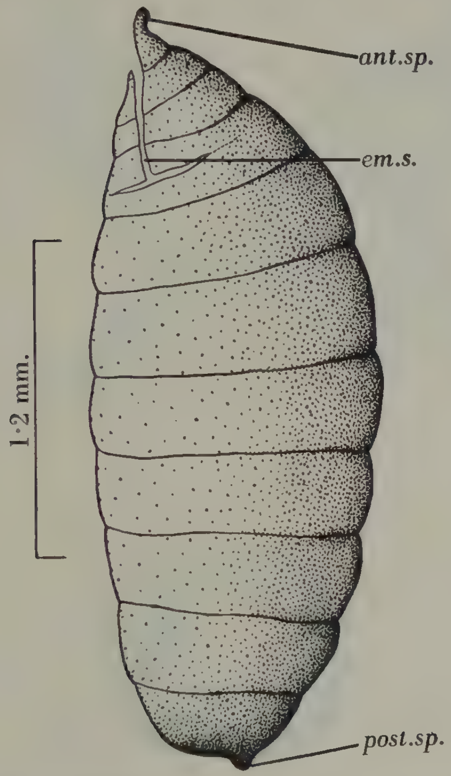
Below the mouth opening and extending laterally on each side is a chitinous labial plate, light brown in colour and with an indefinite margin. It bears a sensory pit—labial palp—on either side of the median line which differs only in detail from that described by Thomas (16) in the third-instar larva of *Opomyza florum* Fabr. and *O. germinationis* L. (17).



Text-fig. 6. Tracheal system of third-instar larva of *Phytomyza atricornis* Mg. *ant.sp.* anterior spiracle; *l.b.* lateral branch; *l.c.* lateral commissure; *d.c.* dorsal commissure; *m.t.t.* main tracheal trunk; *post.sp.* posterior spiracle.

The *cephalo-pharyngeal skeleton* is relatively small in relation to the size of the larva. The mouth hooks possess two teeth of the same shape as those of the second instar. In this, as in the preceding instars, the mouth hooks are unequal in size, the left one being the smaller (Text-fig. 5). The teeth therefore alternate and in whole mounts the mouth hooks may appear to possess three teeth. The hypostomal and pharyngeal sclerites are heavily chitinized and the salivary duct can be seen passing between the hypostomal sclerites, which are connected dorsally by a chitinous bridge. There are no accessory, or dentate sclerites (Text-fig. 3 C). Above the antenno-maxillary plates and on either side below the mouth opening are patches of conspicuous denticles, such as have been described for the second-instar larva.

The intersegmental grooves are covered with rows of denticles which are chiefly confined to the ventrolateral regions of the body wall. There are minute sensory pits on the ventral surface of the thoracic and abdominal segments. Examination of numerous preparations of the third-instar larvae has so far failed to reveal the presence of rudimentary larval thoracic feet such as were described for "numerous *Agromyzidae*" by Keilin (5) in 1911.

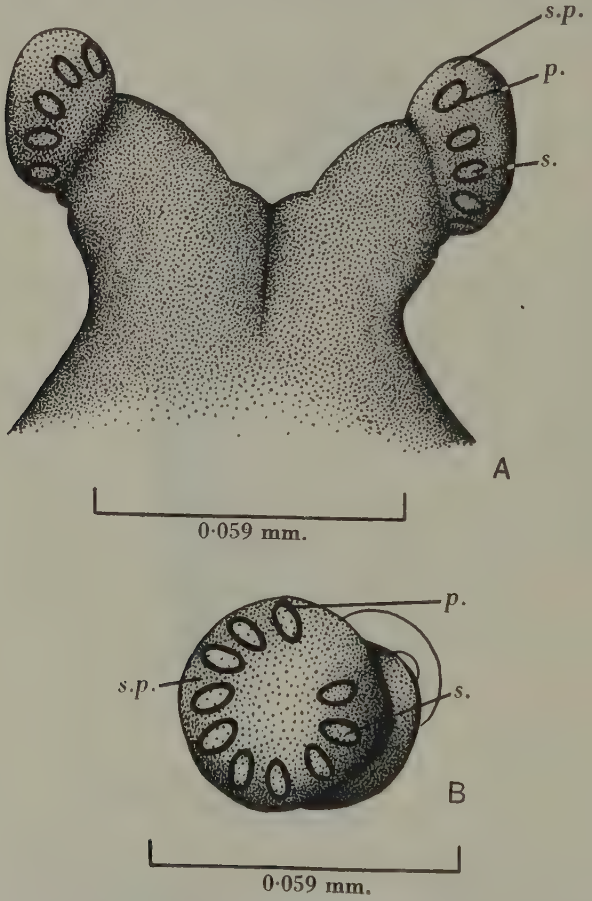


Text-fig. 7. Puparium of *Phytomyza atricornis* Mg. showing emergence slit. *ant.sp.* anterior spiracle; *post.sp.* posterior spiracle; *em.s.* emergence slit.

The third-instar larva is amphipneustic and the tracheal system (Fig. 6) agrees generally with that of the second-instar larva. The spiracles only differ from those of the second instar in the number of pores and digitate processes. The anterior spiracles have from six to ten processes, while the posterior spiracles have relatively fewer, usually from six to nine.

GROWTH RATIO IN THE LARVAE

Growth ratios have been obtained in the larvae of Lepidoptera (2) and Hymenoptera symphyta (10) by the measurement of head width and frons width in the respective groups. By measuring the length of the mouth hooks in each instar an attempt was made to obtain a growth ratio for the larvae of *Phytomyza atricornis*, but no satisfactory result was obtained. This negative result was supported by calculations made from the scale figures given by Thomas (16, 17) for *Opomyza florum* Fabr. and *O. germinationis* L. respectively, and by Steel (15) for *Oscinella frit* L. The fact that there are only three instars in the insects mentioned is against the discovery of a possible growth ratio.



Text-fig. 8. Puparial spiracles. A, anterior spiracles; B, posterior spiracle. Lettering as in Text-fig. 5.

THE PUPARIUM (Pl. XXVII, fig. 6)

The puparium is formed from the integument of the third-instar larva, and, although quite soft at first, soon hardens and becomes rather brittle; possibly the calco-sphaerites present in the larva are utilized in its formation. The puparium (Text-fig. 7) is flattened, elongate and ovoid in outline and cross-section. It varies in length from about 2·1 to 2·5 mm., with an average width of 1·0 mm. There is also great variation in colour at all times of the year, some puparia being creamy white in colour, whereas others are dark brown. Parasitized puparia are generally very dark in colour.

The anterior spiracles (Text-fig. 8 A) which always project through the leaf surface to the exterior are situated at the apex of the puparium on the dorsal surface. The spiracles are directed outwards and forwards, both arising from a common base. Each spiracle is club-shaped and heavily chitinized, and the pores, which are ten in number, are situated round the rim. In the third-instar larva the stigmatic pores are arranged in two irregular rows.

The posterior spiracles (Text-fig. 8 B) are also heavily chitinized, but, unlike the anterior spiracles, are separate at the base. There are ten stigmatic pores arranged round the rim of the spiracle in the form of an incomplete circle. The variation in length of the puparia suggested that it might be possible to separate them into two groups corresponding with the sex. It was found that male flies always emerged from puparia which measured not more than 2·2 mm. long. Female flies always emerged from puparia measuring 2·5 mm., and the majority of puparia measuring 2·2–2·5 mm. also gave rise to female flies.

EMERGENCE OF THE ADULT

The adult fly emerges from the anterior end of the puparium by means of a slit which extends from just in front of the anterior spiracles, round the sides of the puparium, to the metathoracic segment. The act of emergence causes the ventral portion of the puparium to be bent outwards and sometimes detached completely. The detached portion is triangular in shape and carries with it the cephalo-pharyngeal skeleton of the third-instar larva. The anterodorsal part of the puparium is easily detached, having been strained during the process of emergence; this is also triangular, the apex being formed from the anterior spiracles.

DESCRIPTION OF THE ADULT (Pl. XXVII, fig. 3)

Interorbital space and ventral region of face yellow: two subequal superior orbital setae: one to two inferior orbital setae: cheek equal to a third of half the depth of the eye: antennae black; third segment of the antenna quadrate, slightly longer than broad: arista almost bare, scarcely more than twice the length of the third antennal segment: mesonotum entirely black with a light grey bloom: pleural sutures narrowly yellow: four pairs of stout dorsocentral setae: acrosticals absent: femora, tibiae and tarsi dull black: knees yellow, feet brown or black: wings hyaline: second, third and fourth segments of costal vein as 4 : 1 : 1½, veins 2 and 3 subparallel, vein 4 nearly straight: halteres yellow: squamae and cilia dark brown: length 2–2·5 mm.

PARASITES

During some periods of the year it is almost impossible to breed adults of *P. atricornis* Mg. from material collected either in the field or from glasshouses, owing to the abundance of parasites. Parasitized larvae continue to eat through the plant tissue, and in all the parasitized material examined pupation took place. The leaves are thus covered with mines although the parasites are present in the larvae. Parasitized puparia are generally very dark brown in colour, and the fully fed parasite larva can often be seen inside.

Two species have been bred, a braconid and a chalcid, and they are awaiting identification. It is probable that the chalcid is a hyperparasite.

The following figures were obtained from an examination of infested material of the chrysanthemum "Mary Wallace" in December 1934. Out of 115 puparia only seven adult *P. atricornis* Mg. were bred, ninety-eight puparia giving rise to specimens of the braconid. The extent of parasitism was 93·3 per cent. Generally, however, the parasite is not quite as numerous as this.

During the propagating season, when in some districts attacks by the leaf-miner appear to be severe every year and quantities of chrysanthemum "cuttings" are destroyed, parasites have not been bred in large numbers. Later in the season, when the plants are attacked by flies which have bred in wild composites, such as *Taraxacum* sp., *Sonchus* sp. and *Senecio* sp., severe injury to chrysanthemum foliage again occurs, and during this period, which continues from late spring to past mid-summer, the parasite is very common and undoubtedly reduces the number of adult flies in subsequent attacks. Since the parasite allows the larva to form a puparium, it does not, however, effect reduction of blemished leaves, so that its economic value is not great and other means of control must be employed (11).

COMPARISON OF THE LIFE HISTORIES OF *PHYTOMYZA ATRICORNIS* MG. AND *P. CHRYSANTHEMI* KOWARZ

Table IV shows the average time for one generation of *P. atricornis* Mg. as described herein and for the closely related *P. chrysanthemi* Kowarz (see p. 613) as described by Smulyan.

Smulyan's experiments were carried out at an average temperature of 16·3° C. The breeding experiments with *P. atricornis* Mg. were carried out at an average temperature of 18·0° C.

Table IV

	P. atricornis Mg. days	*P. chrysanthemi* Kowarz days
Time elapsing between emergence and oviposition	1	1½
Incubation period of egg	4	5
Larval period	10½	13
Pupal period	11½	14
Average length of one generation	27	33½

SUMMARY

1. The synonymy and distribution of the chrysanthemum leaf-miner are discussed.

2. The host plants are enumerated, and it is shown that the attacks on chrysanthemums decrease by August, although the flies are still common on other Compositae.

3. A description and comparison of the morphology of the three larval instars is given, and the method of feeding of the larva is discussed.

4. The puparium and emergence of the adult fly are also described, together with an attempt to separate the puparia into sexes by measurement.

5. Some degree of natural control is secured by the parasitism of a braconid on which a chalcid is probably hyperparasitic.

6. A table is included comparing the developmental period of *Phytomyza atricornis* Mg. and *P. chrysanthemi* Kowarz.

ACKNOWLEDGEMENTS

The writer wishes to record his thanks to Dr H. W. Miles (Advisory Entomologist for the North-West Province) for advice and encouragement on various problems connected with the investigation and for the use of Figs. 1–5 on Pls. XXVI and XXVII. He is also grateful to Mr J. E. Collin for information on the synonymy of the species, and to Mr N. J. Macpherson, Lecturer in Horticulture, Lancashire County Council, and Mr T. D. Taylor, Head Gardener, University of Manchester Experimental Grounds, for material and facilities for observation and experiment.

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EXPLANATION OF PLATES XXVI AND XXVII

PLATE XXVI

Fig. 1. Chrysanthemum leaves showing typical injury by *Phytomyza atricornis* Mg. ×0·75.

Fig. 2. Chrysanthemum leaf showing injury by larvae and adults of *Phytomyza atricornis* Mg. The arrow denotes larva exposed by removal of leaf surface. ×2·9.

PLATE XXVII

Fig. 3. Adults of *Phytomyza atricornis* Mg. ×11.

Fig. 4. Egg of *Phytomyza atricornis* Mg. exposed by removal of leaf surface. ×12·5.

Fig. 5. Adult of *Phytomyza atricornis* Mg. at rest on leaf. Feeding punctures and mine of larva also visible. ×3·2.

Fig. 6. Puparia of *Phytomyza atricornis* Mg. ×7·2.

(*Received 3 January* 1936)

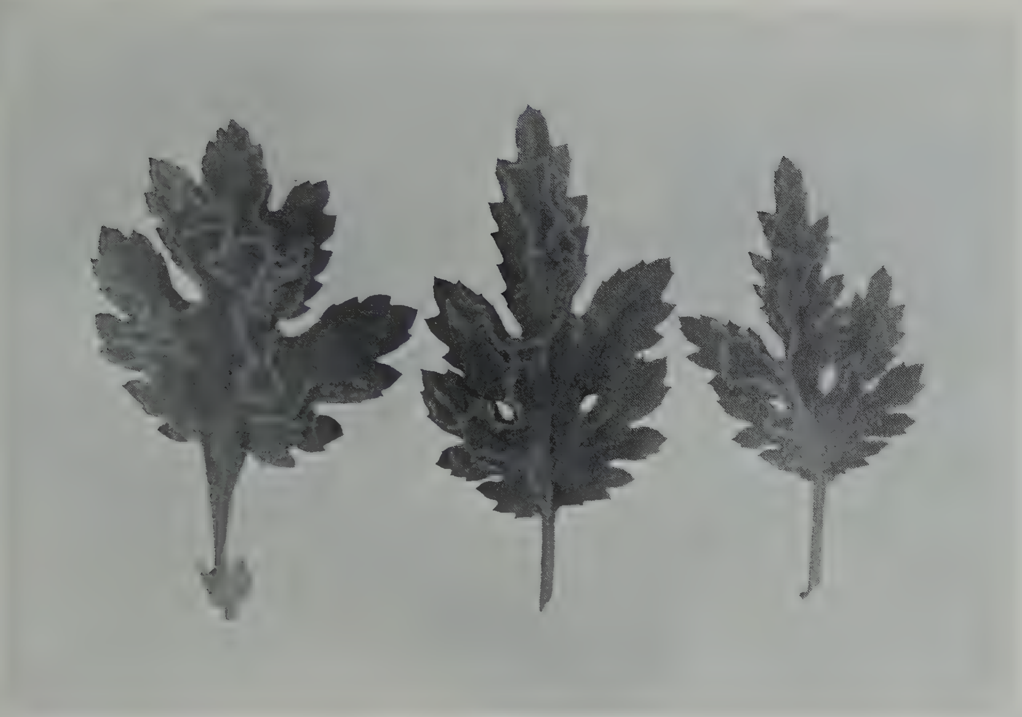

Fig. 1

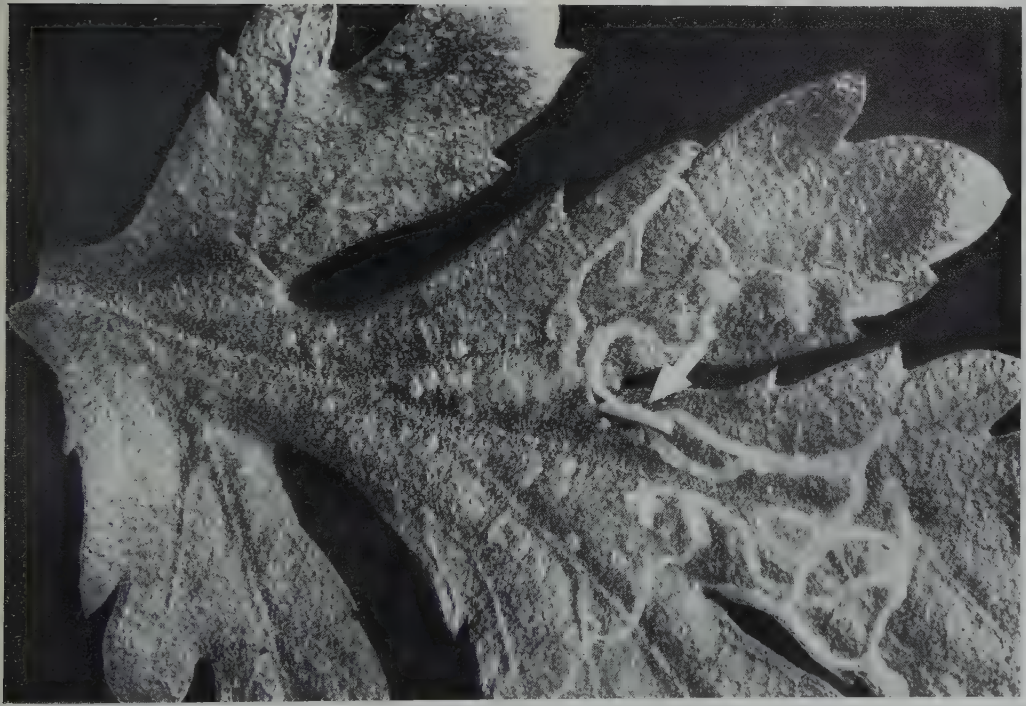

Fig. 2

COHEN—The Biology of the Chrysanthemum Leaf-Miner, *Phytomyza atricornis* Mg. (Diptera: Agromyzidae) (pp. 612–632)

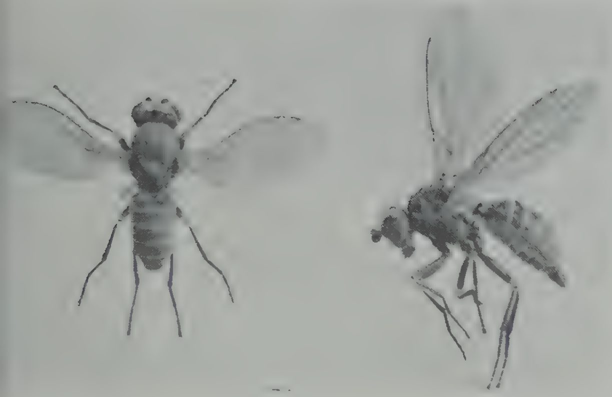

Fig. 3

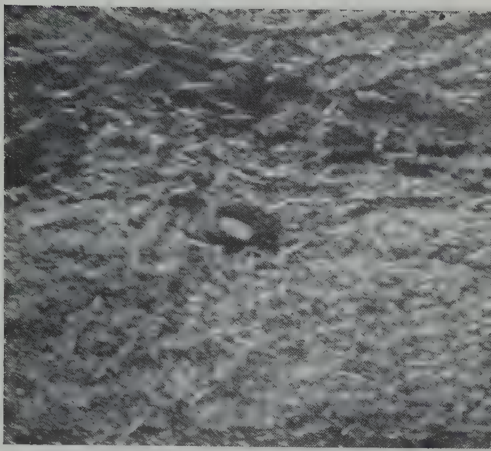

Fig. 4

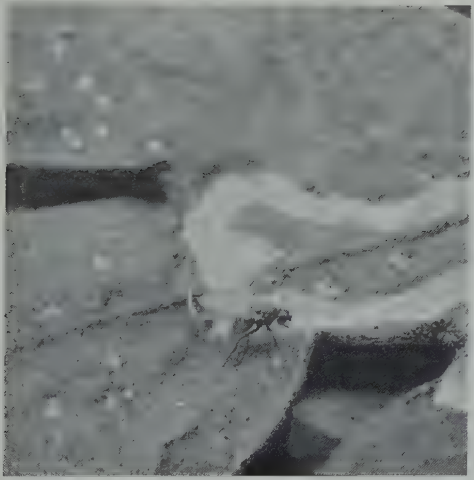

Fig. 5

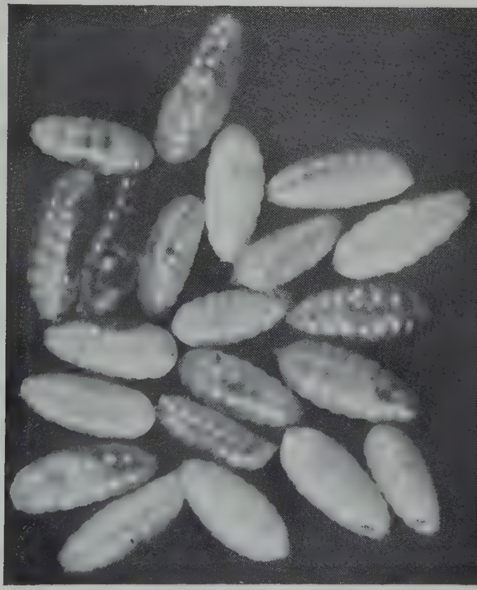

Fig. 6

COHEN—The Biology of the Chrysanthemum Leaf-Miner, *Phytomyza atricornis* Mg.
(Diptera: Agromyzidae) (pp. 612–632)

ON THE OCCURRENCE IN ENGLAND OF THE PEAR FRUIT SAW-FLY, *HOPLOCAMPA BREVIS* KLUG.

By I. THOMAS

School of Agriculture, Cambridge

(With Plate XXVIII and 3 Text-figures)

CONTENTS

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I. Introduction; distribution of the species

There appears to be no record of the occurrence in England of the Pear Fruit Saw-fly, *Hoplocampa brevis* Klug. In 1934 pear fruitlets injured by larvae of what appeared to be this species were brought to the notice of the writer by Messrs K. V. Cramp and L. F. Clift, the Horticultural Organizers for Cambridgeshire. These specimens were obtained from a garden in Cambridge. Only a few pears were infested and no saw-flies were reared from them. In 1935 a careful watch was kept on these trees and a few females of *H. brevis* were beaten from the open blossoms. Later, a nearby garden was found to have a heavier infestation, and it was possible to make a preliminary study of the biology of the species in this country.

In certain continental countries this saw-fly has long been recognized as an important pest of pears. In 1914 Shtchegolev (7) recorded the species as causing great damage around Alushta in the Crimea and along the river Katcha, and in 1924 Falcoz (3) recorded from the environs of Vienne en Dauphiné 25–30 per cent. of fruit sometimes attacked. Observations on the life history of the saw-fly in Denmark were made by Bovien(1) in 1928, and a short description of the adult is given by Enslin(2) who states that the species is present throughout Europe.

From the literature it appears that the pear is the only host plant attacked to any extent by *H. brevis*. Sprengel(8) states that it appears to be only an occasional pest of *Prunus* sp. According to Enslin(2), Brischke records the larva from pears, but Hartigs states that the larva lives in roses; Enslin, however, points out that this latter statement is a misconception and quite incorrect.

II. The adult

The species is smaller than *Hoplocampa testudinea* Klug. Enslin states that the size is 4 5 mm., and gives the following diagnostic characters (Pl. XXVIII, fig. 1): Mesonotum brown, spotted black. Stigma light yellow, the base brownish. Head and antennae brownish yellow without black colouring apart from the eyes. Thorax reddish yellow, the mesonotum somewhat darker with dark streaks on the individual sclerites and on the base of the scutum. Metanotum black, mesonotum densely punctured, almost matt. Legs yellow, wings hyaline with yellow veins. Dorsal surface of abdomen black, ventral surface yellow.

Two females were beaten from pear blossom on 18 April and three females from the same tree on 23 April. No males were caught; Enslin states that the males are very rare, so that it is possible that the species reproduces parthenogenetically. On 25 April three females were enclosed with pear blossom, and one of these immediately commenced egg laying. During oviposition the female faces the bottom of the truss grasping the receptacle firmly below the sepals; a small incision is made with the saws and the ovipositor is thrust into the receptacle at an oblique angle. The egg is inserted immediately beneath the epidermis in a blister-like cavity. The blister is generally very easy to find and is usually present just below the level of the sepals; the egg becomes visible on lifting up the epidermal layer of the blister. The egg is not laid beneath the epidermis of the *upper layer* of the receptacle as with *H. testudinea*.

III. The egg

The egg (Text-fig. 1) is white and translucent, and measures 0·7 by 0·26 mm. It varies only slightly in shape from that shown in the text-figure. The hatching period was not observed. On examination of infested pears on 11 May a number of minute larvae were found; these were at first taken to be first-instar larvae, but on measuring the head capsules and determining the number of instars according to Dyar's Law, it is thought that they were probably in their second instar. It is therefore probable that the hatching period is short and in the region of 5 or 6 days as determined by Bovien (1).

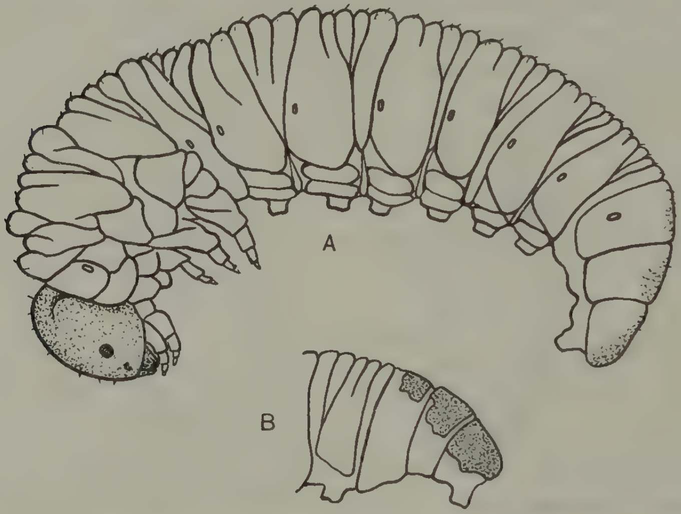
IV. THE LARVA AND DESCRIPTION OF DAMAGE

The second-instar larva is white and translucent, and about 2·5 mm. long. Just after ecdysis the head is very light brown, but before the next ecdysis its colour changes to very dark or almost black. There is a dark brown chitinous plate on the dorsum of each of the last three abdominal segments; on the eighth segment the plate is small, but on segments 9 and 10 it covers almost the entire dorsal surface. The claws are black and there are black markings on the sides of the thoracic legs.

Text-fig. 1. Eggs of *Hoplocampa brevis* dissected out from pear blossom. ×63.

From the damage observed it appears that the larva bores from the blister directly into the young fruitlets. The typical damage is a blackening in this region and a minute hole from which there is a slight exudation of frass. On 11 May three second-instar larvae and two third-instar larvae were found. Further examinations of infested fruit were made on 18 May, 27 May and 6 June. On 18 May one third-instar larva and four fourth-instar larvae were present, and on 27 May there were six fourth-instar larvae and one larva in the fifth instar. On 6 June only two larvae were found, both in the fifth instar, so that by this time most of the larvae were fully fed and had dropped to the ground. Bovien (1), working in Denmark, gives 10 July as the date of entering the ground, but Falcoz (3), working in France, states that damaged pears drop after the exit of larvae about 30 May.

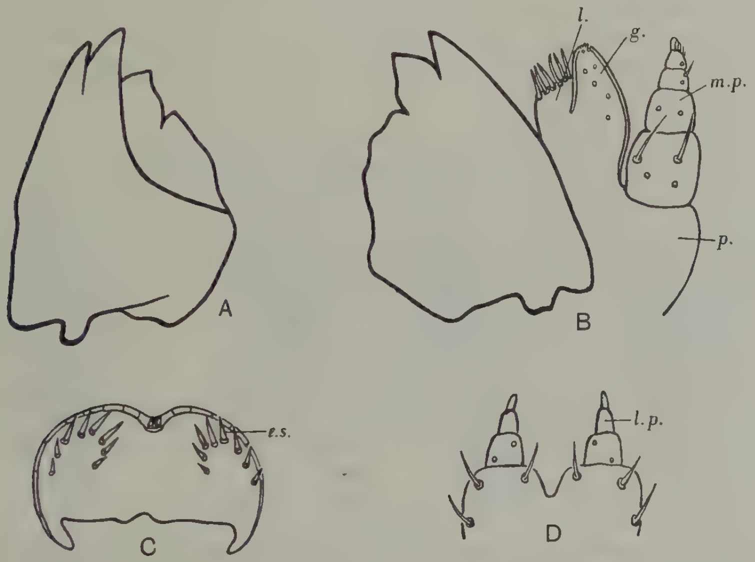
Larvae may leave the original fruitlet infested, but it was not determined how many fruitlets could be damaged by one larva. The injury in the later stages is similar to that done by the second-instar larva described above; holes are bored in the side of the fruitlets and the inside of the young pear is eaten out (Pl. XXVIII, fig. 3). The larva grows fairly rapidly, and in the third and fourth instars it closely resembles the second-instar larva. The head just before ecdysis is very dark brown, and there are chitinized caudal tergites on the last three abdominal segments (Text-fig. 2B). In the fifth instar (Text-fig. 2A), however, the head is light brown and there are no conspicuous caudal tergites. In this instar the larva is fully grown and is about 7–9 mm. long. The body and the legs are pale green and the claws brown. Although there are no caudal tergites there is a slight darkening of the dorsal surface of the anal segment, a very slight darkening of the ninth segment and a slighter hardly noticeable darkening of the eighth segment.



Text-fig. 2. A, fifth-instar larva of *Hoplocampa brevis* taken out of cocoon. ×15·5. B, caudal segments of fourth-instar larva of *Hoplocampa brevis*. ×15·5.

The *head* is subglobose and very similar to that of *H. flava* described by Miles *et al.*(5). The *frons* is pentagonal and has two dark brown diverging streaks. The *antennae* are small and inconspicuous. The *clypeus* is short and broad. The *labrum* (Text-fig. 3 C) has a straight edge where it is attached to the clypeus and is emarginate on its lower edge; it bears two groups of stout epipharyngeal setae (*e.s.*).

The mandibles (Text-fig. 3 A and B), the tips of the maxillary palpi and the end of the labrum are dark brown. The eyes are black. The *mandibles* are heavily sclerotized and dentate; the dentations of the left and right mandibles are dissimilar and closely resemble those of *H. flava*. The *maxillae* (Text-fig. 3 B) are also very similar to those of *H. flava*; the *galea* (*g.*) is a fleshy palp, and the *lacinea* (*l.*) bears a group of conspicuous setae. The *palpiger* (*p.*) bears a four-segmented *maxillary palp*. The *labium* (Text-fig. 3 D) bears six setae on its lower surface, and its palps (*l.p.*) are three-segmented.



Text-fig. 3. Mouth-parts of *Hoplocampa brevis*. × 95. A, left mandible; B, right mandible and maxilla; C, labrum; D, labium; *e.s.* epipharyngeal setae; *g.* galea; *l.* lacinea; *l.p.* labial palp; *m.p.* maxillary palp; *p.* palpiger.

The thoracic and abdominal segments 1–7 are annulated, and prominent spiracles occur on the prothorax and on abdominal segments 1–8. The thoracic legs are not quite as long as those of *H. flava* and have a brownish fused tarsus and tarsal claw. Prolegs occur on abdominal segments 2–7 and 10.

Table I gives the widths of the head capsules of the various instars compared with those of *H. flava* as given by Miles *et al.* (5) and with those of *H. testudinea* as given by Miles (4). These suggest that the first-instar larva was missed.

Table I

Average width of head capsules

	H. testudinea	*H. flava*	*H. brevis*
Instar I	0·392	0·376	?
Instar II	0·550	0·546	0·48
Instar III	0·786	0·683	0·68
Instar IV	1·106	0·915	0·91
Instar V	1·526	1·051	1·13

V. The cocoon

The fully fed larvae fall to the ground at the end of May and early June. On 23 July thirteen cocoons were recovered from the soil beneath a pear tree at depths varying from 2 to 8 in. which corresponds closely with the depths recorded by Miles (4) for *H. testudinea* and by Petherbridge *et al.* (6) for *H. flava*.

The cocoons (Pl. XXVIII, fig. 2) are made of a dark brown parchment-like material, and in a sandy soil they are rough-coated owing to the sand particles adhering to their surfaces. They vary in length from 5·9 to 6·2 mm. and in width from 2·9 to 3·3 mm. Cocoons examined on 11 January 1936 still contained viable larvae, so that the species probably over-winters as a larva.

VI. Summary

Larvae of *Hoplocampa brevis* Klug., the Pear Fruit Saw-fly, have been found attacking pears in two gardens in Cambridge. In some continental countries this is a serious pest of pears; this is the first record of damage by this pest in England.

A preliminary study of the biology of this species has been made, and the egg, larval stages and cocoon have been briefly described.

The writer is indebted to Dr H. W. Miles for the identification of the saw-fly and for helpful criticism.

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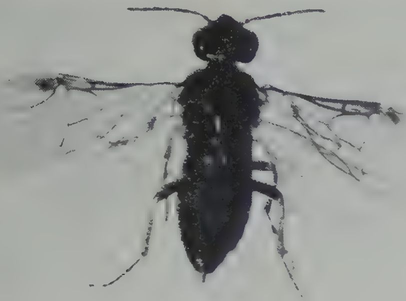

Fig. 1

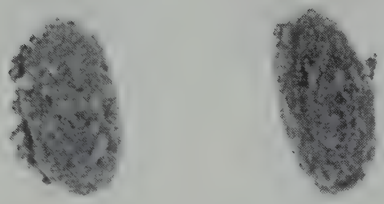

Fig. 2

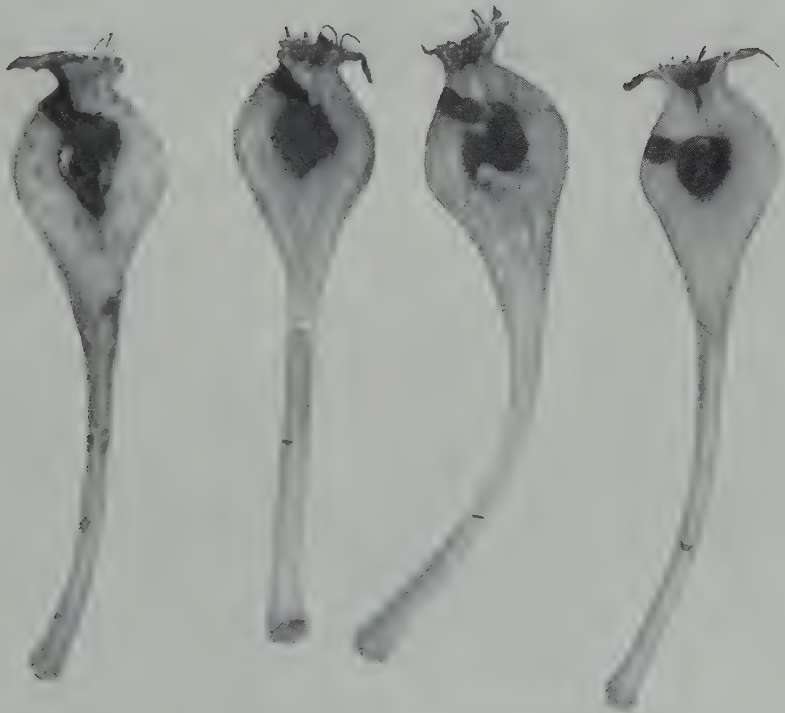

Fig. 3

THOMAS—On the Occurrence in England of the Pear Fruit Saw-fly, *Hoplocampa brevis* Klug. (pp. 633–639)

(5) MILES, H. W., THOMAS, I. & HEY, G. L. (1933). On *Hoplocampa flava* L., the plum saw-fly. *Ann. appl. Biol.* XX, 722.

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EXPLANATION OF PLATE XXVIII

Fig. 1. Adult ♀ of *Hoplocampa brevis*.

Fig. 2. Cocoons of *Hoplocampa brevis*.

Fig. 3. Pear fruitlets damaged by larvae of *Hoplocampa brevis*.

(*Received* 12 *January* 1936)

DAMAGE TO WHEAT BY *HELOPHORUS NUBILUS* F.

By F. R. PETHERBRIDGE and I. THOMAS
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(With Plate XXIX and 3 Text-figures)

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INTRODUCTION

THE only previous records of damage to wheat by *Helophorus nubilus* F. are those of Petherbridge (3) in which he states: "I have found the larvae of this beetle injuring wheat in Cambridgeshire and Lincolnshire." The former record was made in January 1922 when a few larvae of this species were found injuring the young shoots of wheat on a gravel soil on the University Farm, Cambridge; the latter refers to larvae found feeding on the central shoot of wheat plants at Drainage Farm, Bourne Fen, Lincolnshire, on 12 January 1923. In 1921 (2) larvae of *Helophorus* sp. were reported eating the growing points of wheat in Yorkshire, and these were probably also *H. nubilus.*

Fowler (1) says of the adults "common and widely distributed in England...prefer sandy places near water;...often found in heaps of decaying rubbish or under leaves, or even by sweeping herbage, far from water." Sharp (4), quoting Hope, states that "*Empleurus* (*Helophorus*) *nubilus* is widely distributed in the British Islands and is no doubt common over much of Western Europe. *Nubilus* is often found at the roots and stalks of cabbages; the water held in the leaves of the plants being quite sufficient to saturate the ground around and satisfy the insects. I have watched the same insects for eight or nine weeks at the same plant, and never knew their numbers during that time increased or diminished."

During the present investigation the larvae were most abundant in sandy soils and were not found near water.

DISTRIBUTION AND EXTENT OF INJURY

On 16 February 1934, three larvae of *H. nubilus* were sent to us by Dr G. H. Bates, Agricultural Organizer for West Norfolk, from a field near Hunstanton in which wheat plants were dying. On 21 and 26 February careful examinations of this field were made. The soil was sandy and the tilth very loose. In part of the field where the injury was more severe, the previous crop had been rye grass and clover, and in another part after sainfoin the attack was fairly bad. In a small patch 20 × 10 yd. the wheat was good and no *Helophorus* larvae were found, and on this area, previously the site of a stack, the wheat followed potatoes. A detailed examination of the worst part of the field showed that about 80 per cent. of the plants had been injured and about half of these had been killed. Occasional wireworms and Frit-fly larvae were also found, but the bulk of the damage had been caused by the larvae of *H. nubilus*. Another examination on 16 April showed that the larvae had caused further injury and were almost fully fed, twenty larvae and one pupa being found. Shortly afterwards this field was ploughed up, as there was not sufficient plant to warrant the expectation of a satisfactory crop.

A number of fields in which wheat was being eaten off were then examined in various parts of Norfolk and Cambridgeshire. Several fields where a large number of plants had been damaged or killed by *H. nubilus* were found in East Norfolk, three at Bixley about 3 miles south-east of Norwich, one at Blofield 7 miles east of Norwich, and one at Bradenham about 18 miles west of Norwich. At Hevingham, 8 miles north of Norwich, two fields were suffering from a moderate attack. In other fields occasional larvae of *H. nubilus* were found. In all the badly attacked fields the previous crop had been red clover (or trefoil) and rye grass. At Hevingham also the crops attacked were after either red clover and rye grass or trefoil and rye grass; in a neighbouring field, separated only by a lane, the wheat, which was partly after beans and partly after sugar-beet, was very good and no *Helophorus* larvae were found.

In Cambridgeshire, wheat badly attacked was found near Shelford 3 miles south of Cambridge, near Bottisham 5 miles west of Cambridge, and at Melbourn 9 miles south-south-west from Cambridge. In the first two instances the wheat followed sainfoin. The wheat at Melbourn, visited on 8 March, was of interest in showing the effect of previous treatment on the damage caused by this pest:

Section A. After sainfoin. Ploughed in October and drilled the last week in November. Here the wheat was badly damaged.

Section B. After cole seed. Ploughed partly in December and partly the first week in January and drilled about a week later. Here the wheat was good and no larvae of *H. nubilus* were found.

Section C. After wheat. Ploughed in September and drilled the first week in October. Here the wheat was good and very little damage due to the larvae could be found.

Section D. After wheat. Ploughed in October and drilled the first week in December. The whole of this section was suffering from attacks of the larvae of *H. nubilus*, the damage being severe in patches, and everywhere there was a moderate amount of injury.

We can offer no satisfactory explanation of the enormous differences in attack in this field. It will be seen that wheat following wheat was badly attacked, but with early ploughing and drilling the attack was hardly noticeable. An attack after cole seed appears unlikely, as this plot was sown shortly after section D.

On examining the fields at Shelford a second time, on 22 March, great difficulty was experienced in finding larvae of *H. nubilus*, although they were found without much difficulty on 28 February. The field at Bottisham was examined again on 23 March and after a search of 1½ hours not a single larva was found; several had been found here on 28 February. Larvae were also difficult to find at Melbourn on 5 April. On examining the fields at Blofield, Hevingham and Bradenham on 20 March it was not so easy to find the larvae as during the previous search, but it was obvious from the amount of damage that a large number had been present. It was after this date that twenty larvae and one pupa were found at Hunstanton.

Several instances of injury to wheat by *H. nubilus* larvae also occurred in Norfolk and Cambridgeshire in 1935. The injury was not as severe as in 1934 and generally occurred in association with damage by Frit-fly larvae after a rye grass and clover ley. One case of serious damage after a sainfoin ley was noted in Cambridgeshire. This occurred in a field of wheat, 9 acres of which had previously been sugar-beet and 6 acres sainfoin. The wheat after sainfoin was a very thin plant and damage on this section was severe, but the wheat after beet looked well and no *Helophorus* larvae were found.

A case of considerable damage to wheat after trefoil was also noted on a very light soil at Ixworth, Suffolk.

TIME AND NATURE OF INJURY

Damage is caused by the larvae during the winter months, very little, if any, occurring in 1934 after the middle of April. On examining the plants without moving the soil some of them appear as though damaged by Frit-fly and others as though injured by wireworm. Where the plants are close together a wireworm usually kills all the plants in that part of the drill length where it has fed, with the result that all the leaves turn yellow; the larva of *H. nubilus*, on the other hand, usually feeds on some plants neglecting neighbouring ones and does not necessarily kill the plants, with the result that the area where it feeds contains a mixture of green and yellow leaves.

Injury to the plants by these larvae may be found in several different positions (Pl. XXIX):

(1) Below the first node. Occasionally the stem is completely severed. With a firm tilth plants may send out fresh rootlets and re-establish themselves.

(2) Just above the first node. A small hole is often found in this position. This is smaller than the average wireworm hole. In very young plants holes may be found in the coleoptyle. Often the central shoot is eaten through, and this is the type of injury that resembles Frit-fly damage.

(3) Between the first node and the base of the highest expanded leaf blade.

(4) Just above the base of the highest expanded leaf blade. Here the leaf of the central shoot is damaged or completely severed. Holes may also be found in the base of the expanded leaf blades.

In the early stages of this enquiry it was by no means easy to find the larvae even when they were present in fairly large numbers. They are not readily seen in the soil especially when small, as they are almost brown in colour, and it is only very occasionally that specimens are found feeding with their heads inside the plant. The following is a satisfactory method of finding these pests. Insert a narrow trowel near the plants and prise them up very gently, watching carefully for the larva the whole time. Often the larva may be seen to fall to the bottom of the hole and if covered with soil is then difficult to find. If no larva is seen on lifting the plants the soil around them should be gradually removed and examined.

The tilth of the wheat field is one environmental factor which appears to be very important in its effect on the severity of the injury caused by the feeding of these larvae. This is also true of other pests which attack wheat in the early stages. In many of the fields examined the tilth was very loose, and at the time of the attack each plant consisted of one shoot and primary roots. Under these conditions the plants were easily killed, if eaten through below the first node. Occasionally parts of a field had a firmer tilth, and here the plants had commenced to tiller and were sending out secondary roots. In such positions many of the plants recovered from the damage caused by *H. nubilus*, and when eaten through below the first node the secondary roots enabled the plants to establish themselves, although usually the outer leaves had turned yellow. Weather conditions during 1934 made it difficult for farmers to make a firm tilth for wheat on light land, and consequently in many fields, which were comparatively free from pests, the wheat grew badly during the early stages and produced a thin crop.

NOTES ON LIFE HISTORY

We were unable to find eggs and cannot say when the larvae start to attack wheat. In 1923 larvae were found attacking plants on 12 January. In 1935 we found them in four fields in north-west Norfolk on 24 January, and at this date a number of plants showed typical *Helophorus* damage, suggesting that the larvae had been feeding for some time. In 1922 they were found on 31 January and in 1934 on 16 February; at the latter date the damage was extensive. In 1934, in order to study the injury to the plant, larvae were brought back from time to time and put in pots in which young wheat plants were growing. These pots were kept on the roof of the building. On 9 April, one pot was examined and contained two pupae and seven larvae. A search in the field near Hunstanton on 16 April revealed the presence of one pupa about 1 in. below soil-level and twenty larvae. Some of the larvae were fully fed and one was near pupation. Most of these larvae were found in the top inch of soil. On 6 May 1922, five pupae and one larva were found on a wheat field on the University Farm. These were placed in a pot of soil and the first beetle was found on 18 May.

In 1934 pots of wheat kept out of doors and in which larvae had been placed were examined on 26 May. Ten adults were easily found (as they were crawling about on the top of the soil), but no pupae or larvae were present at this date. On 29 May no beetles were found after a 3 hours' search in the field near Hunstanton, but on 27 July one adult was found in the grass growing on the side of the hedge as the result of a 2 hours' search.

The following table gives the dates of finding the specimens present in the collections in the British Museum and the Insect Room at Cambridge:

Month	No. of beetles found	Month	No. of beetles found
January	0	July	1
February	1	August	4
*March	0	September	3
April	1	October	1
May	3	November	0
June	5	December	0

The species over-winters in the larval stage, and the above observations suggest that there is only one generation per annum and that the adults normally live from May till October. The specimens found in February and April suggest that a few of the beetles may live through the winter.

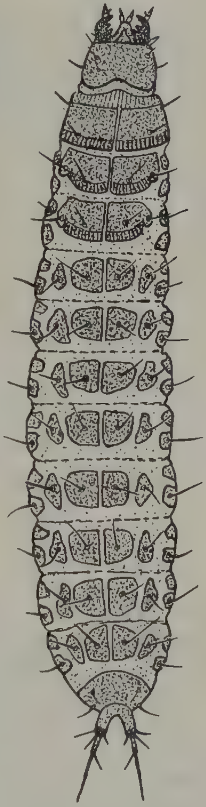
DESCRIPTION

(*a*) *The larva* (Text-fig. 1)

In the final instar the larva, when cleared and mounted, is about 7·3 mm. long (excluding the length of the anal cerci) and has a maximum breadth of about 1·5 mm. It is widest in the region of the fourth, fifth and sixth abdominal segments; and tapers gradually towards the head end and more abruptly towards the tail which terminates in two long anal cerci. The colour varies from dark brown, early in the year, to creamy white, when fully fed; at the later stage the characteristic dark brown chitinous plates bearing setae on the thoracic and abdominal segments show up very distinctly.

The brown *head* is about 0·75 mm. wide and is narrower than the prothorax. Behind each antenna are a group of six ocelli.

The *antenna* (Text-fig. 2 A) consists of a basal and second segment of about the same length and a small terminal segment bearing four setae at its apex. At



Text-fig. 1. Larva of *Helophorus nubilus* (× 13).

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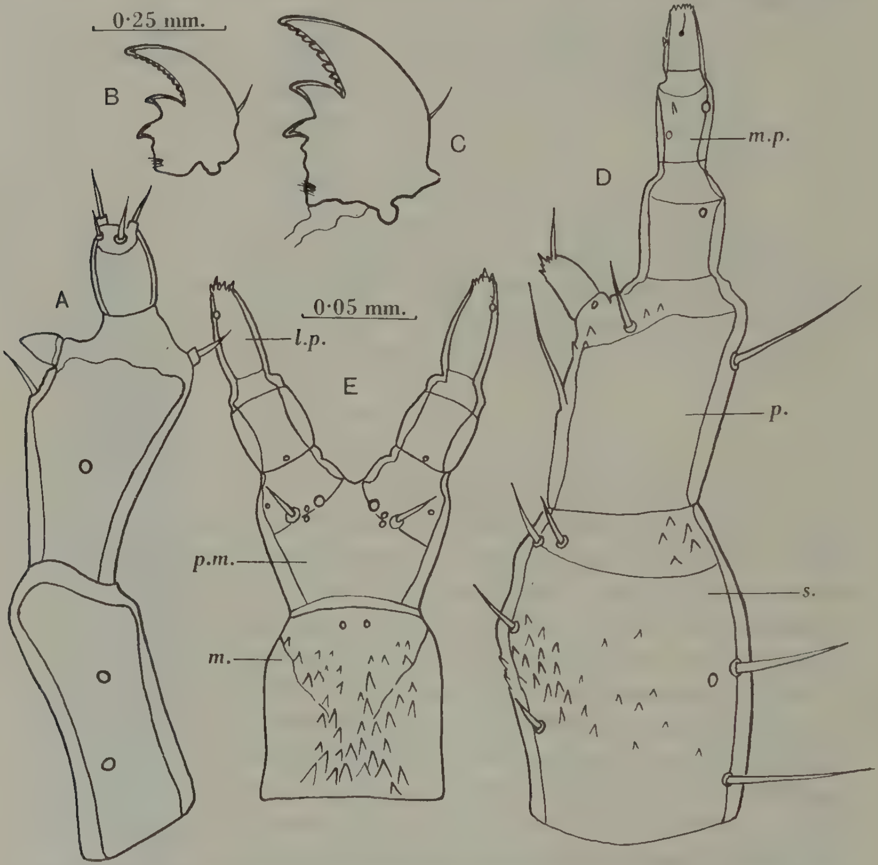
* In mid-March 1936 two adults and two larvae of *H. nubilus* were found in rye grass and clover turf which had been ploughed in during the winter.

its distal end the second segment bears a large sensory papilla and a large seta on either side.

Each *mandible* (Text-fig. 2 B) has three teeth, a long serrated apical tooth with two smaller hooked teeth behind. The serrations on the apical tooth which number about ten are sometimes worn down. Near the inner basal angle is a small bunch of fine setae and on the outer edge is a stiff spine.



Text-fig. 2. Mouth-parts of larva of *Helophorus nubilus*. A, left antenna. B, mandible. C, mandible of *H. rugosus*. D, right maxilla, dorsal view. E, labium, dorsal view. *l.p.* labial palp; *m.* mentum; *m.p.* maxillary palp; *p.* palpiger; *p.m.* prementum; *s.* stipes.

The *maxilla* (Text-fig. 2 D) consists of a three-jointed cardo (not shown in figure), a large stipes (*s.*) with minute chitinous denticles on its dorsal surface and a smaller palpiger (*p.*) bearing a three-jointed maxillary palp (*m.p.*) and a single small palp on its inner surface.

The *labium* (Text-fig. 2 E) consists of a large mentum (*m.*) with chitinous denticles on its dorsal surface similar to those on the stipes and a bilobed prementum (*p.m.*), each lobe bearing a two-jointed labial palp.

The *labrum* has a short median conical process with a larger lobe on either side bearing five ribbon-like papillae.

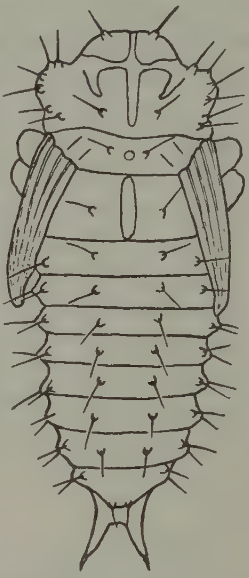
Thorax. The prothorax is narrower and larger than the meso- and metathoracic segments. The scutum is brown and heavily chitinized with a dividing line down the centre which extends throughout the thoracic and abdominal segments. The chitinized scutellar plates on the meso- and metathoracic segments are not as large as on the prothorax and do not cover the whole of the dorsal surface of the segment. Each of these plates bears two long setae and a number of smaller ones. The short legs each terminate in a single claw and bear a number of small setae.

Abdomen. There are nine abdominal segments and with the exception of the last each bears a number of chitinous plates. From the dorsal surface there are visible two large median plates, a smaller one on either side of these and two pairs of lateral plates. There are five plates on the ventral surface, a large median plate with two smaller ones on either side. The ninth segment bears a large chitinous plate covering the whole of the dorsal surface and a smaller plate ventrally. Distally there are two long anal cerci about one-seventh of the total length of the body; each consists of three segments, a wide basal segment bearing three setae at its distal end, a second narrower segment having one seta and a third long narrow segment bearing at its apex a long pointed spine.

(*b*) *The pupa* (Text-fig. 3)

The soft white pupa is about 4·3 mm. long and about 1·5 mm. wide in the region of the prothorax. It bears a series of long bristles arising from prominent conical tubercles. The head, which bears three pairs of bristles, is bent beneath the prothorax and cannot be seen from the dorsal surface. The prothorax is large and broad and has a longitudinal groove in the middorsal line. It bears eight pairs of bristles situated mainly on its dorsolateral margin. The mesonotum is small and narrow, and bears a small circular protuberance on the middorsal line and a long bristle on either side. The metanotum is much larger, has a median longitudinal groove, and carries a pair of bristles similar to those on the mesonotum. The wing pads extend to about the base of the second abdominal segment.

There are nine abdominal segments visible from the dorsal surface. Segment 1 has one pair of dorsal and one pair of epipleural bristles; segments 2–8 each have one pair of dorsal, one pair of pleural and one pair of epipleural bristles. On segment 8 the epipleural bristles may be missing. Segment 9 terminates in two large long lobes each having a smaller pointed lobe at the end.


Text-fig. 3. Pupa of *Helophorus nubilus* (×15).

Summary

Helophorus nubilus F. is recorded as a serious pest of wheat in East Anglia. No previous record of serious damage has been made, but slight injury was recorded in 1922 and 1923. Observations in a number of widely separated fields show that the intensity of attack varies with the previous cropping and a serious attack occurs most frequently after rye grass and clover. The larva causes most damage when the tilth is loose.

Observations on the life history suggest that the species has one generation per annum. Eggs were not found, and the earliest damage to wheat was observed in January. Pupae were found in late April and early May, and adults were bred out in the laboratory at the end of May. The larval and pupal stages are described.

The authors are indebted to Dr G. H. Bates and Mr J. C. Mann of the Norfolk County Agricultural Staff, and to Mr G. W. Channon, formerly of the Cambridgeshire County Agricultural Staff, who reported insect attacks on the wheat fields visited.

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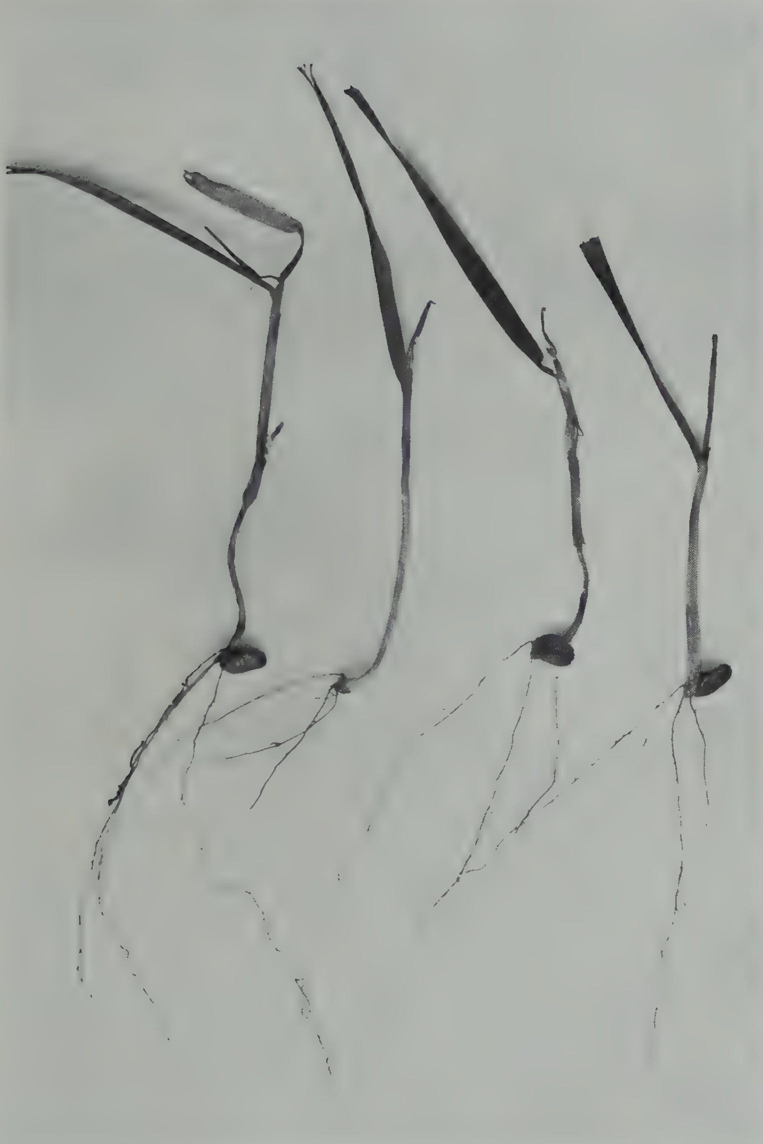
EXPLANATION OF PLATE XXIX

Wheat seedlings injured by larvae of *Helophorus nubilus* F.

(*Received* 19 *October* 1935)



PETHERBRIDGE AND THOMAS—DAMAGE TO WHEAT BY *HELOPHORUS NUBILUS* F.
(pp. 640–648)

THE COMMON RUSTIC MOTH, *APAMEA* (*HADENA*) *SECALIS* L., ATTACKING WINTER CEREALS

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IN February 1934 the writers' attention was called to a field of wheat seriously attacked by a caterpillar which proved to be that of the Common Rustic Moth, *Apamea* (*Hadena*) *secalis* L. (*didyma* Esp.; *oculea* Guen.). There are no previous records of serious injury by this pest in this country, but it is possible that the "young wheat moth caterpillars" described by Ormerod (5) in 1894 were this species. At the time they were thought to be caterpillars of the genus *Miana*, but moths were not bred. Larvae of some species of *Miana*, particularly *M. strigilis*, closely resemble those of *Apamea secalis*, and the caterpillars described by Ormerod were possibly the latter species.

Damaged plants were sent to Miss Ormerod on 21 February 1894 with the following note: "I only find them in lay ground. Where I ploughed it in fallow in the same field the wheat looks well, but where these maggots are they are destroying the whole crop. They eat the heart clean out of the corn." Ormerod states: "The young wheat plants sent showed the serious nature of the damage. This was in various stages—as the whole of the centre of the little plant being cleared out; or the central shoot being bitten off; or the mischief being then in progress. In most instances the little brownish backed caterpillar was set head uppermost, either in the middle of the plant, with moist morsels of material in the cavity, apparently composed of 'frass' (or the rejected results of feeding), or sometimes supported along the back by one of the sheathing leaves, so that it could gnaw away the more central part at convenience. The caterpillar, however, appeared to have an especial preference for so arranging itself in the central shoot, or emptied sheathing leaf of this part of the young plant, that it formed a cylindrical shelter for each larva."

In 1918 (1) it was reported that the larva of a species of moth probably *A. secalis* was found in Shropshire, Hampshire and Wiltshire feeding internally in the base of stems of oats and wheat. Attacks were especially noted on ploughed up pasture.

In 1920 (4) this species, together with *Miana strigilis* Clerck., was found injuring wheat, oats and rye in Shropshire and attacking wheat in Carmarthen. In 1921 (4) it was found attacking wheat in Cambridgeshire and oats and rye in Shropshire and Essex. This report states: "Damage due to these insects either on the increase or becoming more generally recognized. *A. secalis* the chief but not the only species."

There are a number of records of damage to cereals, and particularly rye, by these caterpillars on the continent. In 1913 Lind *et al.* (2) found them in May and June, and in one locality at the end of April.

Shtchegolev (7) records this pest as occurring in unusual abundance in parts of Russia in 1923 and causing considerable injury to winter sown rye.

On 16 February 1934, Dr G. H. Bates, Agricultural Organizer for West Norfolk, sent from a field at Shernborne specimens of wheat plants damaged by caterpillars which proved to be *A. secalis*. This field, which was visited on 21 February, was situated next to a grassy drove and was also adjacent to a strip of land, about 20 yards wide, partly planted with trees and partly covered with grasses. The field was almost bare, and only about 10 per cent. of the wheat plants remained. There was a certain amount of injury due to the larvae of the beetle *Helophorus nubilus* and Frit-fly, but the bulk of the injury was caused by the caterpillars of *Apamea secalis*.

The soil was a light sand, the tilth very loose, and the uninjured wheat plants were not tillering or forming secondary roots. Apart from insect attacks the wheat plants were very poor. This field had been down to red clover and rye grass the previous year and was ploughed up in October and sown with wheat. In March the wheat was ploughed up and the field resown with barley. When the barley was examined on 16 April, no damage from *A. secalis* could be found. The neighbouring field also adjoining the strip of trees and grasses contained winter barley; here some of the plants had been killed by the larvae of *A. secalis*, but the damage was not serious.

Caterpillars were also found attacking wheat in a field about a quarter of a mile away. Subsequently a number of fields were examined but generally only a few caterpillars were found. On 21 March, however, Dr Bates recorded on light land at West Acre (west Norfolk) a complete failure of wheat due to a combination of an attack by larvae of *A. secalis* and drought conditions. On 15 March 1935, Mr A. W. Punter, the Assistant Agricultural Organizer for East Suffolk, sent caterpillars of *A. secalis* from a wheat field at Somerleyton, Nr. Lowestoft, and stated that the larvae had nearly ruined the crop on a 12-acre field.

The injury in every case was similar to that described by Ormerod; the caterpillar was generally to be found feeding at the base of the central shoot, which was sometimes eaten away, leaving only the sheathing leaves; in the later stages of the attack the sheathing leaves were also eaten and the whole plant destroyed.

Caterpillars brought back to the laboratory fed voraciously when caged with young wheat plants. They crawled up the plants and entered between the sheathing leaves and the central shoot; then they moved down the plant to a position near soil-level where they began to feed. Eventually plants were eaten through about $\frac{3}{4}$ in. above ground level. Four larvae caged on 20 March had eaten off fourteen wheat plants by 27 March. Moths were bred out early in July, and on the night of 22 July moths were taken at light traps set in a drove near the badly attacked wheat field at Shernborne. The above attacks occurred on wheat taken after a ley.

Grasses are the normal hosts of this caterpillar, and it seems probable that eggs are laid in the ley before ploughing and that the caterpillars feed on the grasses before attacking the wheat. Rostrop & Thomsen(6) record several severe attacks by this pest on rye and barley, and they noted that the damage was generally more severe where fields adjoined grassy droves. They also record damage by young larvae in the autumn and injury to the developing ears of barley in the summer. Meyrick(3) under the name *Hadena didyma* Esp. gives its habitat "in stems of grasses, September–May".

The caterpillar varies in general body colour from pale yellowish green to olive green. The head and prothoracic plate are brown, and there is a brown dorsal plate on the last abdominal segment. The middorsal line of the larva is of the general body colour, on either side is a wide irregular brown line broken at the segmental margins, below this and just above the spiracles is a fainter line of the same colour; this appears only as brown pigmented areas in some specimens. The spiracles are well-marked black rings, and the crochets of the prolegs are of the uni-ordinal mesoseries.

SUMMARY

An account is given of damage to winter cereals by larvae of the Common Rustic Moth (*Apamea secalis* L.).

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DAMAGE TO PANICLES OF *ALOPECURUS PRATENSIS* L. BY *APAMEA SECALIS* L.

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(With Plate XXX and 1 Text-figure)

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I. INTRODUCTORY

THE object of the present paper is to describe and place on record an unusual type of damage due to the larvae of *Apamea secalis* L. For the sake of completeness, it has been thought desirable to give a brief account of the insect, its distribution, life history and economic importance.

The senior author is responsible for the more purely entomological sections; the junior for the Irish observations and the first association of this peculiar type of panicle injury with the larvae of this insect.

Description of moth and caterpillar. The moth, *Apamea* (*Hadena*, *Trachea*) *secalis* L. (*didyma* Esp.; *oculea* Guen.) or the Common Rustic, belongs to the family Noctuidae. It is exceedingly variable in colour and several colour forms have been named. The fore-wings may be blackish or brownish with a white reniform mark, or brownish with a darker central band, or reddish ochreous with the front marginal area reddish brown and the outer margin bordered with the same colour. The hind wings are fuscous. A specimen reared from a larva causing the unusual type of damage at Harpenden, Herts, in 1935 is illustrated on

Pl. XXX, fig. 1. The expanse of the wings is slightly more than 1 in. (28–35 mm.).

The caterpillar is green with three reddish lines on the back. The head and plate on the first ring are pale brown, as is also the plate on the last ring.

Geographical distribution. This insect is common everywhere in the British Isles, and abroad it is found eastwards as far as western China and Japan.

Life history, host plants and usual type of damage. The moth is on the wing in July, August and September, and there is one generation during the year. The caterpillars are to be found from September until the following April and May. Pupation takes place in late April, May and June below ground.

The caterpillars feed in the stems of many grasses (especially *Festuca*, *Dactylis* and *Poa annua*) and on wood-rush, *Luzula* (Juncaceae).

The usual type of damage is the destruction of the shoots of the grass owing to the habit of the larvae of feeding inside the sheaths.

Economic importance. This species has been recorded as a pest of cereals,[1] especially rye, in the following countries: the British Isles, Holland, Germany, Denmark, Norway, Bulgaria, Russia and Japan. In all these cases the damage apparently has been the destruction of the stems between the months of October and early June. Shtchegolev (3) states that the eggs were found in chains at the beginning of September near the leaf sheath. In captivity the caterpillars hatched in about a week. The immature larvae were found within the stems as late as June, and the portion of the plant above the point of attack withered. He also states that the pupal stage occurs in the surface soil and lasts between 20 and 28 days. The first pupa was found in this case on 21 June. Pittioni (2) includes this moth in a list of noctuid pests found feeding on honeydew in Germany.

II. UNUSUAL TYPE OF DAMAGE TO PANICLES

Description. The result of this particular type of damage is that the heads of the grass have a "moth-eaten" appearance (Pl. XXX, fig. 2). Oviposition has not been observed by us, but it appears that eggs must be deposited within the sheath of the flowering shoot at a relatively early stage of its rapid vernal development. The larva was repeatedly found during April and May 1926 feeding within the sheath upon the soft young panicle, its attention being confined at this stage to the florets, the rachis not being bitten. In no case was more than one larva found in one sheath. When the flower head "shot", the larva allowed itself in a few instances to be carried thereby out of the sheath, but in the large majority of cases it remained in the sheath when the head shot and proceeded to make its way downwards to the uppermost node. In a number of cases this journey was observed to take several days, and the larva could be found resting at a point about half-way down the sheath. Arrived at the base of the sheath, the larva took a final meal by eating through the soft basal portion of the culm and then emerged by piercing

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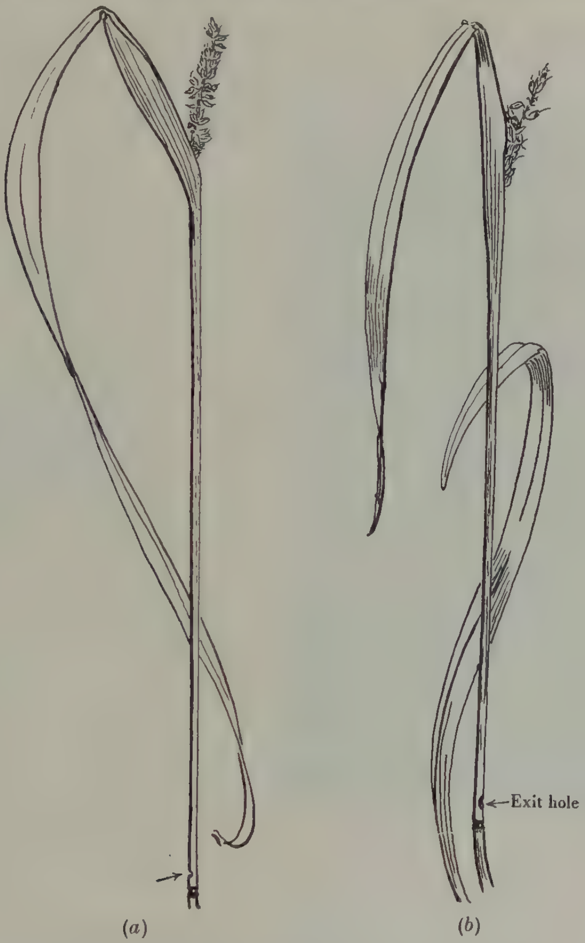
[1] See F. R. Petherbridge and I. Thomas, *Ann. appl. Biol.* 1936, XXIII, p. 649.



Text-fig. 1. (*a*) Showing point of last meal by the caterpillar before departure from stem; (*b*) showing exit hole of larva.

the sheath at a point immediately above the node (Text-fig. 1 *a* and *b*). Pupae were collected from the soil about the bases of injured plants, and these were bred out along with pupae from larvae collected in leaf sheaths; the mature moths obtained proved in all cases to be *Apamea secalis*.

The panicle was never found entirely denuded of florets, but the immediate result of the severing of the culm was the death of the whole head with any uninjured florets which might remain.

Occurrence in Northern Ireland. Isolated instances of the peculiar form of damage described were noted in Northern Ireland during the years 1922–5, but no investigation was made until 1926. In that year the incidence on wild grasses was much heavier than normal. So far as could be observed, the attack was confined to wild wayside and waste land grasses or, alternatively, to small clumps and patches in cultivated gardens, and it was noticeable that grasses growing in sheltered positions (often in partial shade from trees) were most commonly injured. Damage of the kind described was noted upon *Alopecurus pratensis*, *Anthoxanthum odoratum* and *Dactylis glomerata*. This probably does not indicate special preference on the part of *Apamea* so much as convenience, these three species being available at the season required. *Anthoxanthum*, in this area, is the first wild grass to flower and usually does so towards the end of March. Isolated heads of *Alopecurus* may be found during the first week of April, and this species is at its peak florescence about the middle of May. Cocksfoot is usually next in order of flowering, but only a few heads appear during May. As between *Anthoxanthum* and *Alopecurus* there may well be some preference for the latter in view of the bitter taste of *Anthoxanthum*; certainly in Northern Ireland *Alopecurus* is much more attacked than any other species. In the years 1927–34 scattered instances were to be found each spring, but in none of these seasons was the attack as heavy as that of 1926 when it was estimated that 1 per cent. of wild *Alopecurus* plants in the Jordanstown district of South Antrim were damaged.

English occurrence. On 12 May 1935, a few flowering culms of meadow foxtail growing at Harpenden were opened by R. F. Barnes in an attempt to find the caterpillars. The flower heads at this time were still mostly enclosed in the leaf sheaths. Several caterpillars were found, one per culm, and in each case the developing panicles showed signs of damage. Unfortunately only in one case was the caterpillar extracted without injury. This individual was reared by feeding it on developing panicles extracted from grass culms. On 5 June it buried itself in soil and formed

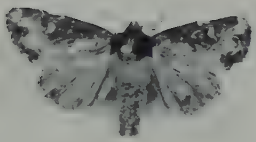


Fig. 1

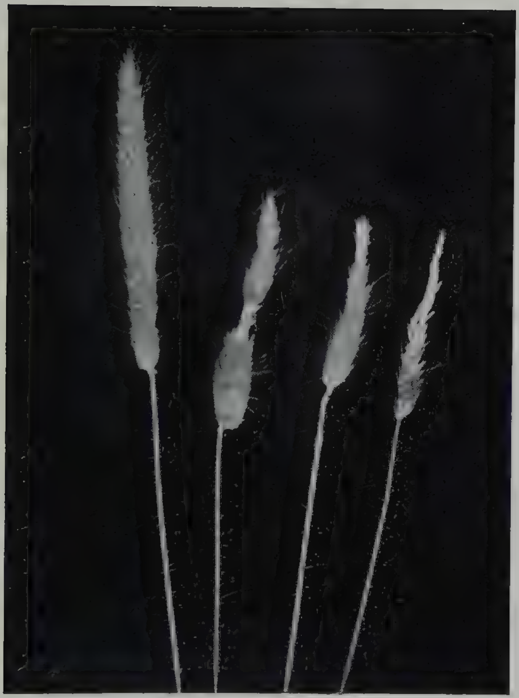


Fig. 2

BARNES and MERCER—Damage to Panicles of *Alopecurus pratensis* L. by *Apamea secalis* L.
(pp. 653–657)

an earthen cocoon. The caterpillar was observed to be still quite active within the cocoon on 8 June. On 15 July the adult *Apamea secalis* emerged.

This type of injury to the panicles was quite noticeable on parts of Harpenden Common in 1935 as soon as the foxtail heads appeared. Whether this particular type of damage only occurs in certain seasons and in such years replaces to some extent the more usual type of injury, or whether it normally occurs and supplements the usual dying off of the stems is an open question.

Mention should be made of the fact that similar damage to the panicles of Timothy grass (*Phleum pratense*) is caused by the larvae of the flies *Amaurosoma flavipes* and *A. armillatum*. For an illustration of this injury reference may be made to a figure published by R. S. MacDougall (1).

III. SUMMARY

A brief description of the moth and caterpillar stages of *Apamea* (*Trachea*) *secalis* L. is given, as well as notes on its geographical distribution, life history, host plants and economic importance. The normal damage is the withering and death of the stems of the grasses owing to the caterpillars feeding within the sheaths.

An unusual type of damage is described and figured. This consists of the destruction of the flowering culms and head, exceedingly similar to that caused by the larvae of *Amaurosoma flavipes* and *A. armillatum*. This damage has been observed over a number of years in Northern Ireland, and in 1935 at Harpenden, Hertfordshire.

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EXPLANATION OF PLATE XXX

Fig. 1. *Apamea secalis* L.

Fig. 2. One healthy flower head of Meadow Foxtail grass; three flower heads attacked by caterpillars of *A. secalis*.

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REVIEWS

The Land Now and To-morrow. By R. G. Stapledon. Pp. xvii + 336, 14 figs. and 2 maps. London: Faber and Faber, Ltd. 1935. 15*s*.

To write any adequate review of this stimulating but highly controversial book would require a volume almost equally large, for *The Land Now and To-morrow* is not only the outcome of fervent enthusiasm, wide knowledge and great experience but really contains a lifetime's philosophy. So widely ranging is it in its scope, so many dreams does it contain and so much ardent idealism that, although amply documented by technical argument and data, it leaves one rather breathless and bewildered. Everyone who reads the book will probably feel strongly that something must be done, and that quickly, but many may also feel that the situation is so complex and involves so many divergent policies and vested interests, that there remains considerable doubt as to the best point at which to break practically into the rapidly revolving and vicious circles which have made the land now and seem already to have committed it for to-morrow.

In the first three chapters the author considers the agricultural background, stating his case in general terms which he summarizes in "A Warning and a Request to the Reader" as follows: "...the logical sequence is to deal first with land improvement and reclamation. The nature and extent of the rural facilities that should be provided must largely be determined by the scale of productiveness to which the land in different regions can be brought—and therefore treatment of this subject must follow after land improvement. The human aspects of my argument—landmindedness and urban recreation—will then be dealt with more fully." In the following six chapters the author, accordingly, deals with the resources of the land and, in eight further chapters, with financial, technical and social methods of land reclamation and improvement. These chapters contain the solid technical and scientific premisses upon which Prof. Stapledon's further argument depends. In the succeeding five chapters the more human problems are considered and, although the author gives rein to his idealism and dreams his dreams, he remains constantly awake to reality and keeps his feet firmly planted on the land.

In Chap. XXIII Prof. Stapledon describes one of his dreams, "My National Park", in some detail. "I propose to assume that I have been asked to organize a municipal (national) park on an area of about 150,000 acres, and that I have been given the necessary authority and power to select and purchase a suitable block of country anywhere I like in Wales. I will now briefly describe the park as I have envisaged it." It is a splendid dream and one can not only hope that it may come true but give active support to any steps taken to make it come true. Of the following two chapters the first states the need for an all-embracing survey and enquiry, and the second which, from certain points of view, is one of the most interesting in the book, discusses education and research. The last chapter, containing "Further and Final Proposals", suggests the setting up of an additional State Department, a "Ministry of Lands", the main responsibility of which would be "planning for posterity". One wishes that Prof. Stapledon might become the first Minister. The concluding paragraph of this chapter so well expresses the aims of the book and the ideals of its author that it may, perhaps, be quoted in full.

"I have brought together a large mass of facts; I have expressed many opinions, and made a number of concrete proposals. As I have said before, not one of my proposals is in essence new, and yet in the aggregate they perhaps suggest possibilities of a new and happier England. An England which I fear to many can only be pictured as a figment of the imagination, as something that can be dreamed of perhaps but never made a reality. I am not sure, for the country as a whole is more land conscious than it has been for many a long day. If my book only encourages further dreaming and imagining about the land it will have more than served its purpose, for deep in the inner recesses of man's subconscious mind lies the indelible impression of the land—a heritage which, by greatly dreaming and greatly imagining, could be galvanized into a mighty power, capable of strengthening the conscious endeavours of mankind and directing them along paths leading to creative achievement in the spheres of individual happiness, personal health and social justice."

The book opens with a synoptic contents, closes with an interesting bibliography and a good index and, in a folder, contains two fascinating maps of "My National Park". The photographic illustrations are beautiful, and the format of the book reflects great credit on the publishers.

Perhaps few people who read Prof. Stapledon's book will agree with all his ideas and suggestions, but none will have any doubt of its importance and, after twice reading the book, my own opinion is that it is one of the most fecund and outstanding works of our time. It is really a work on scientific and agricultural sociology, and whilst there are plenty of scientists who can produce useful technical books on agriculture, and plenty of economists and politicians who can write idealistically of society and the land, there are very few men indeed who possess scientific knowledge and social vision based on a humane and fundamental sanity, who love the land and are at one with it, who can dream great dreams and who are lit with that flame of enthusiasm which is the genesis of great causes. Details of Prof. Stapledon's work may and probably will need to be revised in the light of further knowledge and experience; that does not matter. What does matter is that of his knowledge and vision has come a light giving meaning and direction to the blind gropings of our scientific, agricultural and social policies. Prof. Stapledon has something of the quality of a twentieth-century Peter preaching a modern crusade to a scientific Jerusalem in England's green and pleasant land. It is a great emprise, and its success will depend not on academic agreement but on practical support.

WILLIAM B. BRIERLEY.

Genetics. By H. S. JENNINGS. Pp. 351, figs. 70. London: Faber and Faber, Ltd. 1935. 15*s*.

Prof. Jennings is known not only as a distinguished academic teacher and research worker in zoology but as the author of a number of volumes which have deservedly earned a high reputation in the popularization of biological science. To simplify current scientific knowledge so that it becomes intelligible to the educated lay reader without oversimplifying and so falsifying it is a very difficult task requiring a clarity of mind and gifts of exposition and illustration such as few professional biologists possess. Prof. Jennings has been conspicuously successful in this regard, but the present volume is not quite up to his standard.

The author describes his book as "an attempt to present the fundamental features of genetics: those features of which every educated person should have knowledge". One expects, therefore, a wide and judicial survey of the fundamental principles of genetics, with a balanced consideration of their relationships and implications, and the creation of a perspective in which the relative importance and scientific and human values of these features would show hierarchical ordering.

The science has made startling progress in the last three decades and is now capable of a certain tectonic formulation, but it is still in a semi-fluid condition and quite incapable of crystallization in forms having sharp edges and outlines. A reading of this book, however, tends to leave the impression on one's mind of a finished scheme, a neat theoretical and self-consistent structure into which fundamental facts may be fitted like elements in the Periodic Table. In reality, geneticists are faced with a great mass of data which as yet cannot be explained.

Further, genetics simply cannot be considered from either a zoological or a botanical viewpoint only, for it is essentially biological. The author's treatment of his subject, however, is essentially zoological, and this leads to a somewhat distorted perspective giving a false balance of values. Plants, of course, are mentioned but receive scant grace. Polyploidy, for example, which is primary amongst the fundamental features of the genetics of plants, receives entirely inadequate consideration, and even then, one of the examples given is incorrect. This lack of balance due to a too narrow viewpoint is shown in Chap. II on "The Genetic System: its History during the Life of the Individual and in the Production of the Next Generation." Botanical research left this account of the genetic system behind a quarter of a century ago, and the system as described is not only at variance with modern data but renders unintelligible a good many results of practical plant breeding.

Further, the previous writings of Prof. Jennings have shown him to be very critical of the propagandist claims of the more unbalanced and vehement eugenists but, on pp. 281 and 282 for example, in his consideration of human heredity, he himself lapses rather badly from his scientific attitude and makes statements for which there is little or no scientific justification. Our rapidly increasing knowledge of the effect of partial or insufficient nutrition on human development and bodily and mental characterization foreshadows plainly the need for a very considerable revision of our ideas on certain fundamental features of human heredity and makes the author's treatment of this subject seem rather dangerously simple and far too definitive.

Of course, as one would expect, the book as a whole consists of well-selected and logically ordered material, lucidly explained in clear and simple language, and admirably illustrated by apt text-figures and diagrams. One's criticisms are of a general nature, are perhaps indicative of one's own personal point of view and made only because a volume on genetics by Prof. Jennings is a work to be taken seriously. As a book on this subject it far outclasses most other volumes of a similar type.

WILLIAM B. BRIERLEY.

Heredity and Evolution. By A. E. WATKINS. Pp. viii + 243, plates 2, figs. 35. London: John Murray. 1935. 7*s*. 6*d*.

In his preface the author says, "In this book, the principles of genetical science, their scope and the evidence on which they rest have been discussed; the bearing of these principles on the theory of evolution being kept in view throughout", and the book is a good introduction to the modern study of genetics in relation to problems of evolution. The contents follow the usual sequence of Mendelism, variation, cell division, sex determination, linkage, chromosome variation, mutation, selection, species, conclusion, and examples are taken from well-known animals or plants according to which is the better illustration in any particular case. Each chapter closes with a minimum list of references and, at the end of the book, are a short bibliography of general works in English, a glossary and an index.

Considering that the author has not shirked the discussion of current and technically obscure problems, his book is an interesting, up-to-date and, in many ways, successful presentation. Occasionally, in the text, the language is not entirely clear, and the actual arrangement of the subject-matter within the chapters is, perhaps, not always the most straightforward. On the whole, however, the author is to be congratulated on the production of a well-balanced introduction to a difficult subject, and his book is one that should have a wide appeal not only to university students but to educated adults who wish to know of the progress that is being made in this important field.

WILLIAM B. BRIERLEY.

Plant Biochemistry. By W. E. TOTTINGHAM. Pp. v + 219. Minneapolis: Burgess Publishing Co. 1934. $4.25.

Knowledge of the physiology and biochemistry of plants advanced rapidly during the past 30 years and, especially during the last decade, reached a condition when text-book formulation became not only possible but very necessary. The result has been almost a spate of text-books on the subject and, whereas pre-war students were dependent almost entirely on Pfeffer, Jost and Czapek, students to-day are almost bewildered by the choice available. In spite of this the present work is a valuable and welcome addition to this literature. In his preface the author states, that "Recourse is taken to mimeographed form as a means of determining whether or not the material here presented merits more permanent treatment", and he may rest assured that most botanists will think that it does. The book has grown out of a course of lectures given by Dr Tottingham during the last 25 years in the Department of Agricultural Chemistry of the University of Wisconsin, so that the author has had ample opportunity to prune and mould his material and to decide on an adequate and successful mode of presentation.

Following a brief general introduction, the author discusses the materials of metabolism and then proceeds to the photosynthesis and metabolism of carbohydrates, fat metabolism, and the metabolism of nitrogen compounds. He then considers the physicochemical relations of the plant cell, which leads to discussions of the nature and function of enzymes and the chemical aspects of respiration. A chapter follows on salt nutrition and the book closes with a discussion of climatic effects in metabolism. It is all good, straightforward material, clearly and concisely put together, with pages full of C, H, O and N in all the numerical and structural arrangements beloved of biochemists, and with an abundance of diagrams and tables to illustrate the text. As befits an author coming from the agricultural side, the examples throughout the book are mostly taken from plants of economic importance, agricultural or horticultural. The author's treatment of his subject is hard and technical; it is essentially a research presentation with an assumption of considerable physiological and biochemical knowledge on the part of the reader. Anyone, however, who can read Chap. II on "The Materials of Metabolism" with understanding will find no difficulty with the remainder of the work. Literature citations, some of the year 1934, are introduced freely into the text, and at the end of each chapter is a short list of books of reference.

The volume opens with a detailed table of contents, but there is no index, which is a very serious lack: author and subject indices would have added enormously to its value, and it is greatly to be hoped that if, as is desirable, the work assumes a more permanent form, these will be added and the references collected in a terminal bibliography.

The book is a noteworthy contribution to the literature of the vast borderland between chemistry and botany, bringing together and marshalling in orderly array a wealth of data and experience of the chemical processes of plant life.

WILLIAM B. BRIERLEY.

Éléments de chimie végétale. By N. WATTIEZ and F. STERNON. Pp. 729, figs. 62. Paris: Masson et Cie. 1935. Fr. 100.

There has long been need for an up-to-date treatise on plant chemistry, a volume of convenient size, well arranged and with some degree of detail, and containing good bibliographies to give one access to the literature on special problems. The present book meets this need admirably.

Following a preface by Prof. E. Marchal, and a short introduction, the first chapter generally introduces the subject in a brief consideration of the plant cell. Chap. II is concerned with the methods used in plant chemistry, Chap. III with their application in the analysis of elaborated materials, and Chap. IV, which is the remaining three-quarters of the book, with the descriptive study of the elaborated materials. The volume closes with a good index and a table of contents. Each chapter is divided into numerous sections, at the end of which are extensive lists of references, some of which are marred by misprints.

The book impresses one as an excellent piece of work, it is written in clear and concise language and, as a reference text, will be of considerable value to botanists.

WILLIAM B. BRIERLEY.

An Annotated Bibliography of the Low Temperature Relations of Plants. By R. B. Harvey. Pp. ii + 223. Minneapolis: Burgess Publishing Co. 1935. $4·00.

The importance of research on the low-temperature relations of plants is increasingly being recognized, partly because of the light it is throwing on fundamental processes in normal physiology and in diseased conditions and, partly, because of its enormous practical value in relation to the storage and transport of food. Botanical interest in low-temperature relations is, of course, of long standing, Goeppert, Sachs, Pfeffer, Sorauer and other great nineteenth-century physiologists laying enduring foundations. During the past two decades, however, there has been a great concentration of attention upon these problems, and the literature of the subject, written in numerous languages and widely scattered in all kinds of scientific journals, has become unwieldy. Prof. Harvey and his associates have spent many years studying, translating, checking and indexing this literature and have now made their labours conveniently available to others in this volume.

In all, there are 3412 citations, which include publications up to the year 1934, and these are numbered and arranged alphabetically by authors. The titles in languages other than English are given in the original language and in translation and, if the title is not self-descriptive, the nature of the contents is briefly indicated. In addition to the immediate field of plant relations, citations are included on the low-temperature relations of animals when these are helpful in elucidating similar relations in plants. The work closes with an excellent subject index.

Only those who have had experience in compiling a large bibliography from scattered sources will appreciate the devoted labour and scientific acumen required to produce such a work as this. Such bibliographical research, for it is research of a valuable kind, is urgently needed in many fields of biology, and the author may rest assured in his hope that "this bibliography...will so encourage interest in and study of the subject that the research phases may be more quickly advanced". To physiologists and pathologists the work is invaluable.

The book is produced in mimeographed form on one side of the page only, which is very useful, in that current references may be inserted on the blank side. It is bound in a green fabroid cover and, as the Burgess Publishing Co. is issuing a series of books in this style, one might suggest that the author or abbreviated title be indicated on the back so that any particular volume may be identified without removal of the entire series from the bookshelves.

WILLIAM B. BRIERLEY.

Weeds. By W. C. Muenscher. Pp. xvii + 577, 123 figs. New York: The Macmillan Co. 1935. 25*s*.

The study of weeds occupies an important place in the agricultural and horticultural curriculum, and the present volume is a very welcome addition to the literature of the subject. The contents are divided into two portions. Part I, 90 pages, contains four chapters dealing respectively with the dissemination and importance of weeds, weeds of special habitats, the control of weeds, and chemical weed control. The first 36 pages of Part II contain a key to the groups and species of weeds, and the remaining 400 pages contain descriptions and illustrations of weeds arranged according to family. The book closes with a Glossary, a page of Ready Reference Data, 11 pages of Literature References and 25 pages of double-column Index.

Part I is extremely well done, concise, clear and accurate, and it not only summarizes the literature but contains the results of much first-hand observation and experiment. By the skilful use of tables the author manages to pack into it an enormous amount of information.

In Part II, the 500 species described are, of course, to some extent, a somewhat arbitrary and personal selection from the weed flora of the northern United States and Canada. So far as the key has been tested it enables one to run down the species successfully, and it is well and clearly arranged. In the descriptive portion the weeds are arranged in alphabetical order under their scientific names by families, and synonyms are included. For each weed data are given concerning nomenclature, duration, reproduction, dissemination, habitat, range, source, recognition and control, particular emphasis being laid on identification and control. The descriptions are concise yet entirely adequate. A notable feature of the book is the 123 pages of text illustrations which consist of line drawings of weed plants usually including roots, with further floral structure, fruit and seed. These drawings are unusually beautiful and were made from the plants by Mrs Helen Hill Craig. The Glossary is serviceable, the Bibliography whilst not complete is a useful compilation, and the Index is well prepared.

The book is very well printed and produced and seems to be free from errors and misprints. Altogether it is a noteworthy addition to the literature of weeds and, whilst primarily written for North American students, it is a volume that should be in every botanical, horticultural and agricultural library.

WILLIAM B. BRIERLEY.

Ugressfrø (*Unkrautsamen: Weed Seeds*). By EMIL KORSMO. Pp. 175, with 34 plates. Oslo: Gyldendal Norsk. (London: Williams and Norgate Ltd.) 1935. 42*s.*

Prof. Emil Korsmo of Oslo is recognized by all agricultural botanists as one of the foremost authorities on weeds. He has probably done more to place their study on a scientific basis than any other living person, and his book *Unkräuter im Ackerbau der Neuzeit*, published in 1930, is a landmark in the subject. He has now placed botanists still further in his debt by the publication of the present magnificent volume on *Weed Seeds.*

The fruits, and more especially the seeds of plants, are neglected aspects of study and, at need, it is often difficult to find exact descriptions and illustrations, or adequate collections which one may consult. For some 306 species of weed plants, principally those distributed in America, and Europe north of the Mediterranean regions, the author has now removed this disability. The weed flora of Europe and America contains a great number of species, and the term "weed", itself, does not connote any exact type of plant. The author has, therefore, had to select and has mainly concentrated on species the seeds of which are found frequently as impurities in seed material, especially in various species of grains, grasses and legumes.

Prof. Korsmo has, rather astonishingly, arranged his material in ecological groups according to the life forms of the plants. It is difficult to see the reason for this as it has no bearing on the appearance, shape and structure of fruits and seeds, and it results in the irregular dispersal of species of one family throughout the book. Thus *Polygonum* species are illustrated on Plates I, XXII and XXIX, *Ranunculus* species on Plates X, XX, XXIII, XXVII and XXVIII; *Rumex acetosa* is figured on Plate XXIX and *Rumex acetosella* on Plate XXXIV and, on the latter plate, there are, in addition, *Epilobium angustifolium*, *Hypericum perforatum*, *Euphorbia cyparissias*, *E. esula*, *Convolvulus arvensis*, *Roripa silvestris*, *Lepidium draba* and *Linaria vulgaris*. As this book will be almost entirely used for the description and identification of fruits and seeds, a systematic arrangement by families, genera and species would have been much more practical and useful.

The descriptions are given in Norwegian, German and English, arranged in parallel columns under the heading of the species and family. Details are included of the infructescence and fruit of each species, the appearance, size and weight of the seed, the distribution of the weed and the way it is spread through the sale of seed material, and a lettered explanation of the illustration. These descriptions are, in most cases, models of clarity and condensation, and excellent use is made of standardized abbreviations.

The main feature of the book is of course the 34 coloured plates, each containing nine species, and showing the infructescence and fruit, the seed in two planes, transverse and longitudinal sections of the seed, and the seed natural size. These plates can only be praised: they are clear and accurate, giving one exactly what one needs, and the colour reproduction is so good that their perusal is a real delight.

At the end of the book there is an Index of descriptions and illustrations, arranged alphabetically according to the genus and species of the plant, and this is combined with the popular names of the weed arranged in parallel columns in American, Danish, English, French, Dutch, Italian, Canadian, Norwegian, Russian, Swedish and German. This is a very useful feature and should help to standardize the cheerful diversity of popular nomenclature.

The volume is beautifully produced, its publication being made possible through financial support by the Norwegian Government, and it is a book which should be available in every botanical, agricultural and horticultural department. Botanists throughout the world will be grateful for the author's labours and take joy in the fact that they have found so worthy a setting.

WILLIAM B. BRIERLEY.

British Stem- and Leaf-Fungi (*Coeolomycetes*). Vol. I. *Sphaeropsidales.* By W. B. Grove. Pp. xx+488, with 31 text-figures. Cambridge: University Press. 1935. 21*s.*

It is 64 years since M. C. Cooke in his *Handbook of British Fungi* enumerated some 200 species of what are now classed as Coeolomycetes and, although many of these fungi are of primary economic importance and have received a great deal of attention there has, in this country, been published no systematic treatise devoted to them. This is not because the necessity for such a work has been unrecognized but, simply, that these fungi are so difficult to determine and classify that only Mr Grove would have the patience and courage to attempt it. Vol. I of his long and eagerly awaited work has now appeared and it amply fulfils expectations. The present book includes all those British forms of the Sphaerioideae which have colourless or nearly colourless spores, i.e. the Hyalosporae, Hyalodidymae, Hyalophragmiae and the Scolecosporae, dealing with some 50 genera among which are included such economically important assemblages as *Phyllosticta*, *Phoma*, *Phomopsis*, *Cytospora*, *Ascochyta*, and *Septoria*. The remaining Sphaerioideae, i.e. the Phaeosporae, Phaeodidymae, Phaeophragmiae and Dictyosporae, the other sections of the Order Sphaeropsidales, i.e. the Nectrioideae, Excipulaceae and the Leptostromataceae and the other Order within the Coeolomycetes, i.e. the Melanconiales, will occupy Vol. II.

The author has examined microscopically nearly all the species he describes and, where this is not the case, the fact is noted. No cultural and few pathological details are given, for Mr Grove is a mycological systematist of the old school and his diagnoses are purely morphological. The author, of course, recognizes the difficulties in a strict adherence to this point of view which compels him to rely for many of his specific definitions upon a host difference. Of this he remarks: "On the whole, the evidence available in the field tends to show that two allied fungi growing upon two different hosts may themselves be equally different. Therefore, generally, in these pages the host will be made the supreme test." It is a test which, however, he uses with discretion and he does not regard his lists of presumed species in many genera as anything more than an interim catalogue of the known and described forms. The species in the larger genera are arranged on the basis of the host genera in alphabetical order, and described species have not been united unless, in Mr Grove's judgment, convincing evidence is available. Following the diagnosis of each species are the author's critical comments, and these are often exceedingly interesting, since the rather dry and classical humour of Mr Grove is not entirely suppressed. Concerning the excluded genus "*Polyopeus*" on p. 162, for example, the author remarks: "The Petripatellist ought to correlate his museum of freaks with previously known species, before indulging in such a futile orgy of nomenclature." The word "Petripatellist", coined by Mr Grove, is rather good.

After the diagnostic consideration there is an Addendum containing a list of species of the discredited genus *Phlyctaena*, which are merely B spores of the genus *Phomopsis*, and Latin diagnoses of 24 new species described in this volume. The book closes with an Index of Hosts and the genera of fungi found upon them, and an Index of Binomial Names. There are 31 text-figures.

The book is a work of first-class systematic importance, worthy of the author of *The British Rust Fungi*, and it will long remain the standard authority on the subject.

WILLIAM B. BRIERLEY.

Manual of the Grasses of the United States. By A. S. Hitchcock. Pp. 1040, figs. 1696. Washington: U.S. Government Printing Office. 1935. $1·75.

Dr Hitchcock's studies in agrostology have justly earned their international reputation, and the author's lamented death soon after this book appeared is an irreparable loss. Fortunately his work was done and it stands as a splendid memorial of a great man. That a volume of this magnitude should be without a "Preface" is some indication of the author's modesty and scientific detachment, but those who had the privilege of meeting Dr Hitchcock will remember his scientific dominance and his warm humanity. The book opens with the sentence "Of all the plants of the earth the grasses are of the greatest use to the human race", and, although much of the author's life was spent in the most desiccated of systematic study, human values were always in the forefront of his mind.

The first 15 pages are an admirably clear and concise introduction to the uses, distribution, morphology, classification and nomenclature of grasses: not a word is wasted. The next 14 pages contain a characterization of the Gramineae, descriptions of the subfamilies and keys to the tribes, and descriptions of the tribes and keys to the genera. The main portion of the book, 743 pages, contains descriptions of genera and species with keys to the latter. There follow 207 pages of synonymy and a few pages containing notes of unidentified names and notes on persons for whom grasses have been named. The volume closes with a useful glossary and a first-class index.

The manual contains descriptions of all grasses known to grow in the continental United States and in Alaska. It includes 159 numbered genera and 1100 numbered species and, in addition, inserted without numbers and excluded from the keys, many unestablished species. A magnificent feature of the book are the illustrations, which cover nearly all the species and were drawn by Mary Wright Gill, Edna May Whitethorn and Agnes Chase. In every case the specimen from which the drawing was made is cited.

No criticism can be made of this book. It is the work of one who, in its writing, was the greatest living agrostologist, and to its making went knowledge and experience which were unique. One can only be thankful for his labours and glad that he saw their completion. The low price of the volume is phenomenal, and one can but be grateful for the wisdom of the United States Department of Agriculture in placing such a work within the reach of all botanists.

WILLIAM B. BRIERLEY.

S. P. WILTSHIRE.

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ERRATA

Page 588, Table I, under Generation III (3)
for part progeny of summer brood II
read part progeny of summer brood I

Page 598, Text-fig. 3 *for* 1925 *read* 1935

Page 601, line 11
for brownish black-winged *read* brownish-black winged

Page 604, second line from bottom
for R. J. Carrick *read* R. Carrick

VOLUME XXIII NOVEMBER, 1936 No. 4

SOIL CONDITIONS AND THE TAKE-ALL DISEASE OF WHEAT

BY S. D. GARRETT, M.A.

From the Department of Plant Pathology, Imperial College of Science and Technology, London

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I. INTRODUCTION

(a) *The existence of the causal organism in the soil and on the host plant*

IT seems to be generally assumed that certain fungi causing root diseases of plants are able to survive in the soil in the absence of their hosts by living as saprophytes on soil organic matter. Thus it is sometimes suggested, without proper foundation, that the incorporation of organic materials in the soil will necessarily result in the saprophytic multiplication of such a facultative saprophyte, and hence aggravate the disease for which it is responsible. Whilst some parasitic fungi, such as the species of *Pythium* responsible for "damping off" of seedlings, may perhaps be able to carry on an active saprophytic existence in the soil, it has certainly been too readily assumed that all such fungi can do so.

Recent work with *Ophiobolus graminis*, the cause of the "take-all" disease of wheat, suggests that its saprophytic potentialities are confined to a power of surviving, to a rather limited extent, on the dead tissues of plants which it has killed. Thus the fungus has been shown (40) to be actively growing in the soil only in the presence of the living host roots. This conclusion is supported by the experience of this investigation, during which the fungus has never been found to grow through the soil, or indeed to show any activity therein, except along the roots of its host plants. The take-all disease thus spreads actively through the soil only by root contact, and is thus comparable in some respects to the root rots of rubber caused by *Fomes lignosus* and *Ganoderma pseudoferreum*, the ecology of which is now being worked out by Napper in Malaya (54). Again, recent studies by Sanford (50) on potato scab have demonstrated that the spread of *Actinomyces scabies* through the soil is negligible.

The life of *Ophiobolus graminis* in the soil thus alternates between a parasitic or ascendant phase and a saprophytic or declining phase. In the declining phase, the fungus is exposed to competition with the other soil micro-organisms. The relation between *O. graminis* and the other soil inhabitants should thus be regarded from an ecological point of view. *Ophiobolus* is a dominant of its local habitat, the living wheat root. After this habitat has been opened up, however, the initial colonizer is replaced by other organisms. There seems to be a regular succession in diseased wheat roots:

Parasite → secondary parasites → saprophytes.

This succession is frequently revealed by the culture plate, and explains the difficulty experienced by workers on cereal foot rots in isolating the causal agent from a root not in the earliest stages of infection. The succession of micro-organisms is not merely a random one. Thus in the case of the *Rhizoctonia* disease of cereals in South Australia (48), whilst roots in the very earliest stages were full of *Rhizoctonia* mycelium, isolations made a week or so later almost invariably gave a preponderating percentage of a certain species of *Helminthosporium* (possibly the *Helminthosporium M.* of Henry (24)). Later still, other species appeared in the culture plates, *Fusarium* generally predominating.

The alternation between these two phases in the life of the fungus is of particular interest in connexion with the effect of soil conditions upon the disease. It has already been shown (37, 17) that *Ophiobolus graminis* is very sensitive to the soil environment. The effect of soil conditions upon the disease will vary, however, according as to whether the fungus is in the parasitic or ascendant phase, or in the saprophytic or declining phase. In the parasitic phase, spread of the fungus is along the outside of the wheat roots (17). A root apparently becomes infected only where it comes into contact with a piece of fungus inoculum residual in the soil, or with another living root on which the fungus is actively growing. Spread of the disease through the soil thus occurs during the growing season of the plants by root contact. The rate of spread of the fungus along the roots varies widely with the soil conditions, moisture, temperature, aeration, reaction, etc., and growth may even be inhibited altogether. In this case, infection is non-progressive, for the fungus can apparently extend only for a few millimetres longitudinally inside the roots, if growth along the outside be stopped (Garrett, unpublished). This inability of *Ophiobolus* to spread appreciably inside the wheat root thus renders it particularly susceptible to soil conditions.

In the saprophytic phase, *Ophiobolus* mycelium is subject to decomposition by the soil saprophytes (61, 62). Vegetative fungus mycelium does not persist in the soil unchanged; if it is not actively growing, it must be decomposing (58). In this connexion, the long-established practice of bare-fallowing the soil for the control of the take-all disease in Australia and elsewhere assumes a new significance. It was formerly considered that in such periods of bare-fallowing, *O. graminis* disappeared from the soil merely through the absence of its host plants. It now appears more likely that its disappearance is due rather to the actual decomposition of its mycelium by the saprophytic fungi and bacteria of the soil microflora (51). Moreover, the efficiency of the fallow period in controlling the disease is known to be increased by good rainfall, a condition making for general microbiological activity in the soil. Thus it is well recognized in Australia that a wet fallow is more effective in ridding the soil of the fungus than a dry one. It thus appears that those very conditions which at one time encourage the activity of the fungus on the roots of the growing crop, viz. adequate soil moisture, good aeration and high temperatures, may at another time hasten its disappearance in the fallow soil.

Further light is thrown upon this aspect of the question by results which have been obtained by various workers in pot tests with the cereal foot-rot fungi. In such experiments large quantities of cultures of the fungi on cooked cereals were incorporated as inoculum in the soil of the pots, creating conditions ideal for the multiplication of soil saprophytes. The rate of decomposition of such organic inoculum in the soil must have varied considerably with soil conditions, especially temperature and moisture. Following upon the work of Henry (25), it was shown by Garrett (18) that the discordant conclusions of different investigators as to the effect of soil temperature and moisture upon these diseases could be reconciled by taking into account this factor of microbiological decomposition of the inoculum. Whilst high soil temperature and adequate soil moisture might actually increase the activity of the pathogen on and in the host plant, these factors would at the same time be working in the reverse direction by hastening the decomposition of the pathogen inoculum in the soil, and hence decreasing the amount of active pathogen.

The possibility of controlling fungus root rots by the addition of organic manure to the soil to encourage the activity of saprophytes antagonistic to the parasite is not a new idea. Such a suggestion was first put forward by Sanford (49) to account for the control of potato scab by green manuring. In 1927, Millard & Taylor (36) showed that the control of *Actinomyces scabies* by green manuring occurred only in the presence of the saprophytic species, *A. praecox*. In 1929, Fellows (15) reported that the take-all disease could be controlled by the incorporation of organic matter in the soil; he found chicken manure especially efficacious. Of most recent interest in this connexion is the very striking control of cotton root rot (*Phymatotrichum omnivorum*), obtained in Arizona by the use of organic manure in field tests, by King *et al.* (31). These authors support their hypothesis of microbiological antagonism as the mechanism of control, by determinations of soil respiration in the manured and unmanured plots, respectively, and by an actual study of the soil microflora in the plots by the Cholodny slide technique.

The present investigation has thus resolved itself into two parts: (i) a study of the effect of soil conditions upon the rate of growth of the fungus mycelium along the roots, and (ii) an inquiry into factors affecting its decomposition in the fallow soil. Only the first part has so far been completed, and it is this which is presented below.

(*b*) *Soil conditions and the occurrence of the disease*

In South Australia, severe epidemics of the take-all disease are practically confined to the light sandy soils of the mallee areas recently cleared from the virgin scrub of dwarf *Eucalyptus* or mallee, as it is known locally. Such soils generally overlie limestone, and are decidedly alkaline, having a reaction of *p*H 8·0–9·0. On the heavier clay-loam soils, neutra to slightly acid in reaction, the disease is never of serious concern to the farmer.

That take-all is worst on the lighter soils is in agreement with reports from Sweden (43), Germany (20, 52), Austria, Czecho-Slovakia and Hungary (39), Holland,[1] and Schelswig-Holstein (26). It was also a commonplace of field experience in South Australia that the disease was favoured by lack of soil compaction, and by any treatment which opened up the soil, e.g. deep ploughing, ploughing in long stubble, and inadequate preparation of the seed-bed. Thus Griffiths (23) remarks: "...during the recent period of low rainfall years when, with dry conditions and much wind, there has been a considerable drifting of surface soils, it has been noticeable that the take-all infestation has not been on hard places from which the surface soil has drifted, but on the parts where it has accumulated, which, although fertile, remain loose and open for a very long period".

In the second place, these light sandy soils of the mallee areas are always alkaline, generally having a *p*H of 8·0+. This, once again, is in agreement with reports from elsewhere that the disease is favoured by alkaline soils, as observed by Fish (12) in Victoria, by Schaffnit & Meyer-Hermann (53) in Germany, by Rosen & Elliot (46) and by Kirby (32) in the U.S.A., and by Blanchard & Carrera (3) in the Argentine.

An inverse relation between soil acidity and the incidence of take-all on the manurial plots at Woburn has been pointed out by Glynne (19). The well-established custom of spraying with sulphuric acid for the control of the disease in France may thus owe its success not only to the fungicidal action of the chemical upon fungus mycelium with which it comes actually into contact, but also to some effect, temporary at least, upon soil reaction.

Manurial deficiencies of the soil have been suggested by Rosen & Elliot (46) as a factor making for greatly increased susceptibility of the wheat plant. The influence of superphosphate in reducing the amount of take-all was well shown by a disease survey of the manurial plots at the Waite Agricultural Research Institute in 1925 (44). The same conclusion has been reached by Griffiths (23) as a result of general field observation in South Australia. At the same time, superphosphate is an acid fertilizer, and the possibility of a transient effect upon soil reaction in the neighbourhood of the seed and crown of the plant cannot be entirely excluded.

In the experiments to be described below, the effect of these and other soil conditions upon the rate of growth of the fungus along the roots has been investigated, in an endeavour to find out to what extent fluctuations in the severity of the disease may be correlated with the activity of the fungus in the soil.

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[1] Private communication by Dr A. J. P. Oort.

II. METHODS

The fungus *Ophiobolus graminis* grows quite well, though somewhat sparsely, in pure culture on sterilized soil. A much denser growth may be obtained by the addition of glucose and cornmeal to the latter. On unsterilized soil, the fungus makes no growth at all; microscopic examination of the fungus inoculum generally reveals no trace of renewed activity. Even if cornmeal be added to the unsterilized soil, this is at once colonized by other organisms, and any incipient growth of *Ophiobolus* quickly inhibited.

It was thus realized that growth of the fungus through natural soils could only be investigated by the provision of plant roots as a substrate.

The method, which has already been described (17), depends upon the fact that *Ophiobolus graminis* can progress only by growth along the outside of the wheat root, which is, however, penetrated transversely by numerous colourless infection hyphae (microhyphae), given off by the dark-coloured runner hyphae (macrohyphae). Since infection hyphae are put out immediately behind the apices of the surface-growing runner hyphae, the visible extent of the latter may be taken as a measure of internal infection. If growth along the outside of the root be stopped altogether by unfavourable soil conditions, then infection is seldom found to proceed for more than a few millimetres longitudinally inside the root. It is not yet clear why this is so, since growth of the infection hyphae transversely across the root appears to proceed without any difficulty.

To obtain measurements of the growth rate of the fungus in a number of different soils therefore, wheat seeds are planted above agar disks (8 mm. in diameter) of the fungus cut out with a cork borer from the margin of a colony growing on a potato or carrot agar. The seeds are planted with their germ ends in the centre of the inoculum blocks, and covered with moist sand. This arrangement insures that on emergence the roots grow into the soil through the inoculum blocks. After a variable period, the fungus begins to grow down the roots. After some 14–16 days at 20° C., all the plants are washed free of soil, and pickled in 70 per cent alcohol, for subsequent recording of the growth made by the fungus.

It is then possible to estimate the rate of growth of the fungus along the roots under the different soil conditions tested simply by measuring the distance travelled by the fungus from the point of inoculation at the seed. The actual measurements are quickly and easily made under the binocular microscope with 60× magnification. The root system of each plant is floated out in 70 per cent alcohol (the pickling fluid) in a Petri dish, and viewed by overhead illumination over a white background. The dark-coloured runner hyphae are then easily visible. A root is followed down under the microscope until the limit of the runner hyphae is observed; it is grasped with forceps at that point, and the distance from the point of inoculation (at the seed) conveniently read off against a millimetre scale drawn on the white background of the microscope stage. Only the three oldest seminal roots are measured for each plant; these are all put out at the same time and under the same infection conditions. The mean of these three oldest roots then gives a figure for the plant as a whole. The actual distance grown by the fungus along the roots is derived from the mean length of root infected by the subtraction of an arbitrary 3 mm., to account for the proximal portion of root actually in contact with the inoculum block. Some twenty-five to thirty plants are generally measured for each series, to establish a mean figure on a satisfactory level of significance. With practice, thirty plants can be measured in less than an hour; moreover, recording for even 8 hours a day involves very little fatigue, since the runner hyphae are so easily visible.

The method has the advantage of being a quantitative one free from the errors inseparable from methods involving personal judgement on the part of the investigator (e.g. McKinney's (35) method for estimation of foot and root-rot infection). In this respect, it is comparable to the "radial advance" method invented by Gregory & Horne (21) for investigating the activity of apple-rotting fungi. With a leaf-infecting fungus, Bonde (4) has compared the pathogenicity of saltants of *Alternaria solani* by measuring the size of the lesions produced by each under controlled conditions on potato leaflets.

Turning now to the subject of soils and general technique, it was realized that the proposed investigation of the effect of soil moisture, aeration, biological content, etc., on the growth of *Ophiobolus* along the roots would require a degree of control obviously unobtainable in pots under greenhouse conditions. A laboratory technique was therefore worked out, based on the use of glass tumblers as plant containers; these have been already employed by the writer in previous work (17). These tumblers are 3½ in. high by 2¾ in. in diameter, and strong enough to stand hard tapping on the laboratory bench, and autoclaving.

Soils were always air-dried before use, and put through a 1·5 mm. sieve. The saturation capacity of all the soils worked with was determined by the Rothamsted method. It was thus possible by a simple calculation to fill the tumblers with soil at any required moisture content. Whilst uniform conditions in sand and very light sandy soils could be obtained at any moisture content simply by mixing the sand with the correct amount of water, weighing out into the tumblers and tapping down on the bench until well compacted, this method could not be used for loams and heavier soils at a moisture content much above 40 per cent saturation, owing to the soil becoming "sticky" and impossible to manipulate.

For the loams and heavier soils, therefore, another method of filling the tumblers had to be worked out, which would give a cylinder of soil comparatively uniform with respect to texture, aeration, and moisture content. The stock soil used in this connexion was a medium loam from Slough, with a saturation capacity of 50 per cent. A moisture content of 70 per cent saturation having been decided upon as most satisfactory for all purposes, the following method of filling the tumblers was adopted. 55 c.c. of the 70 c.c. of water (or other liquid) required for 70 per cent saturation is poured into the bottom of the tumbler; 200 g. of the air-dry soil is added gradually, tapping the tumbler on the bench the while to avoid the enclosure of air spaces within the soil. After filling, the tumblers are left for some hours, when the moisture is generally found to have risen to the surface layer of soil. The final 15 c.c. are then added. In this way, a fairly uniform cylinder of moist soil is obtained, as can be seen by knocking the block of soil out of a tumbler and breaking it across. When required, soil can be steam sterilized in the tumblers before the second addition of water without gross changes of physical texture; the tumblers are weighed before sterilization and loss of weight made up along with the second addition of water. Frequently, however, a large bulk of soil was steamed in 6-in. pots and then air dried, for subsequent use as and when required.

In seeding the tumblers, five inoculum disks, 8 mm. in diameter and cut from the growing margin of an *Ophiobolus* colony on a vegetable agar, are placed in shallow depressions in the soil made by an 8-mm. glass rod. A presoaked wheat seed is planted above each, with its germ end approximately in the centre of the disk. The whole is then covered with 50 g. of sand at 30 per cent saturation, which is levelled and shaken down by tapping the tumblers in pairs on the bench. A batch of forty to fifty tumblers is inoculated, seeded and covered with sand in three consecutive operations. This not only saves time but makes for uniformity of treatment, since tumblers are inoculated not along but across experimental series, in order to eliminate as far as possible the effect of variations in the inoculum from one fungus colony to another.

The tumblers are then packed on shelves in a large 20° C. incubator, and the experiment left for 14–16 days at this temperature. Higher temperatures of incubation have been avoided as detrimental to the normal development of the seedling roots. The tumblers are kept in darkness, thus ensuring complete uniformity of the environment. Light is not considered necessary, since the plants are there only to provide roots as a substrate for the fungus, and it has already been shown that the rate of growth of the fungus along the roots is, within wide limits, independent of the nutrition and illumination of the seedling (17).

After this period of incubation, the tumblers are removed and the plants washed free of soil. A jet of water has proved to be the easiest method of removing the cylinder of soil, with its five plants, from the tumbler. The washed plants, trimmed of tops and all but the three oldest seminal roots, are then pickled in 70 per cent alcohol for subsequent recording.

Several isolates of the fungus were employed during the course of this work. Owing to deterioration of the fungus in culture, it has been found most simple to obtain a fresh isolate from the field at least once every 12 months. This deterioration in pathogenicity is manifested by a notable decrease in the fungal growth rate; for this reason figures derived from experiments performed at different times are not necessarily of the same order.

In conclusion, it must be clearly understood that, in the experimental section to follow, the quantity measured is the amount of linear growth of the runner hyphae along the roots of the wheat plant; this has been found to correspond closely with the length of root actually invaded by the fungus. How far the observed differences in growth may be attributed to the incidence of soil conditions upon the fungus, and to what extent they may be influenced by possible changes in root resistance, are discussed at the end of this paper.

III. EXPERIMENTAL

(a) *General soil conditions and the growth of the fungus along the roots*

The fungus has been found to grow along the roots best of all in sand; if Slough soil be added to sand in increasing proportion, the rate of growth of the fungus falls off in a fairly regular manner. This may be illustrated by data from a typical experiment (Table I).

Table I

Growth of fungus along the roots

Proportion of soil by volume in sand + soil mixture	0	1/16	1/8	1/4	1/2	1
Growth of fungus in mm.	53	40	16	18	6	5
Standard errors	±1·9	±2·3	±2·4	±1·8	±1·2	±1·4

The differences in growth rate of the fungus shown in Table I are significant with the exception of two, that between 1/8 soil and 1/4 soil, and that between 1/2 soil and soil itself. A decrease in growth of the fungus is thus brought about by addition of Slough loam to sand. This might be attributed to a number of factors, decrease in soil aeration, increase in organic matter and microbiological activity, etc.

A decrease in growth rate of the fungus can also be produced, however, by admixture of a comparatively inert substance, pure china clay, with the sand (Table II). This effect of china clay is most readily interpreted as conditioned by the decrease of soil aeration.

Table II

Growth of fungus along the roots

	Control sand	+1/4 china clay
Growth of fungus in mm.	40	28
Standard errors	±1·0	±1·6

The effect of soil aeration is well illustrated by an experiment in which tumblers were filled with Slough soil at 70 per cent saturation (*a*) in the usual manner, closely packed, described under "Methods" above, (*b*) the soil was mixed with the water by hand into small lumps, and then loosely packed into the tumblers (Table III).

Table III

Growth of fungus along the roots

	Closely packed soil	Loosely packed soil
Growth of fungus in mm.	5	14
Standard errors	±1·4	±1·4

The beneficial effect of soil aeration on the growth of the fungus is yet further demonstrated by experiments on soil moisture content. Since the total pore space of the soil is shared between the soil water and the soil atmosphere, it follows that the lower the moisture content of the soil, the better the aeration, and *vice versa*. In one such experiment, a comparison was made between a soil moisture content of 75 per cent saturation and one of 30 per cent saturation; two different soils were employed. The tumblers containing soil at 75 per cent saturation were filled in the usual way; in the case of those at 30 per cent saturation, the soil was mixed by hand with the requisite amount of water and then weighed into the tumblers (Table IV).

Table IV

Growth of fungus along the roots

	75 % saturation	30 % saturation
Growth of fungus in mm.:		
Soil A	15	25
Soil B	6	12
Standard errors:		
Soil A	±0·9	±0·9
Soil B	±1·2	±2·0

The much more rapid growth of the fungus in the soil at 30 per cent saturation may therefore be reasonably attributed to the better aeration obtaining under such conditions, for it hardly seems likely that water can exercise any harmful effect as such. On the other hand, it may be argued that the low moisture content of the soil has modified the plant roots in such a way as to favour the fungus. This objection, together with certain others of a similar nature, will be discussed at some length later on, under section IV of this paper.

Soil can thus be improved for the growth of the fungus by any treatment tending to promote better aeration. It has been found that a marked improvement may also be effected in two other ways:

(i) by making it alkaline,
(ii) by steaming (except in the case of acid soils).

This is well illustrated by an experiment in which a range of soils at different pH values was prepared by treating Slough soil with calculated amounts of sulphuric acid and sodium hydroxide respectively. The treated soils were kept for 3 weeks at 40 per cent saturation and at 25° C. and then air dried. The tumblers were filled with the different soils at a moisture content of 70 per cent saturation, in the usual manner. The steamed series were steamed *in situ* in the tumblers for 2 hours at atmospheric pressure, the second addition of water being made after cooling, along with that added to make up for water lost during steaming. pH values were determined by the quinhydrone electrode (Table V).

It will be observed that in both the steamed and the unsteamed series, the growth of the fungus increases in a fairly regular manner with rise in pH value of the soil from pH 6·0 to 7·5. The effect of pH is considerably more pronounced in the case of the steamed soils. The effect of steaming is to increase the growth of the fungus except in the two most acid soils; in the case of the soil initially at *p*H 5·55, steaming produces a decrease, which is just not significant, however. Briefly stated, therefore, it appears that the effect of steaming is to enhance the effect of soil *p*H.

Table V

Growth of fungus along the roots

	$+H_2SO_4$					$+NaOH$		
Soil treatment	0·4 %	0·3 %	0·2 %	0·1 %	Un-treated	0·05 %	0·10 %	0·15 %
*p*H value:								
Steamed	6·1	6·55	6·7	6·9	7·15	7·25	7·35	7·5
Not steamed	5·55	5·8	6·3	6·7	7·05	7·4	7·6	7·85
Growth of fungus in mm.:								
Steamed	12	15	25	29	28	31	35	37
Not steamed	16	17	16	18	22	25	26	27
Standard errors:								
Steamed	±1·8	±2·3	±2·0	±1·1	±1·3	±1·4	±1·8	±2·0
Not steamed	±1·4	±2·0	±1·6	±1·7	±1·6	±1·5	±1·4	±1·4

It has been found that the addition of lime and chalk to Slough soil produces substantially the same effect as that of sodium hydroxide. Moreover, sodium carbonate and bicarbonate are as effective in this respect as sodium hydroxide. This is shown by an experiment in which air-dried Slough soil was filled into the tumblers at 70 per cent saturation in the usual manner, but employing, in the place of water, solutions of sodium hydroxide, carbonate and bicarbonate, respectively, of such strength as to give 0·15 g. of sodium hydroxide per 100 g. of dry soil, or equivalent sodium in the case of carbonate and bicarbonate (Table VI).

Table VI

Growth of fungus along the roots

	Control	0·15 % $NaOH$	0·20 % Na_2CO_3	0·32 % $NaHCO_3$
*p*H value	7·25	8·1	8·1	8·1
Growth of fungus in mm.	11	25	23	28
Standard errors	±1·2	±1·8	±2·0	±2·1

The differences between the three alkali-treated soils are without significance.

Tests of the growth of the fungus along the roots in series of natural soils from the field strongly emphasize the importance of soil *p*H as a factor controlling the activity of the fungus. The results of two such tests may be quoted. In the first experiment, seven English soils were employed; the steamed soils were, in this experiment, steamed in pots and then air dried before filling into the tumblers; a second series was not steamed. The saturation capacity of each soil having been previously determined, it was a simple matter to adjust the moisture content of each soil series to 70 per cent saturation when filling the tumblers. The seven soils employed were as follows:

Richmond Deer Park, pH 4·8, a sandy heath soil.
Welsh, pH 4·8, a yellow soil, overlying a slate formation.
Devon, pH 4·9, a red loam.
Slough, pH 6·8, stock soil.
Chelsea Physic Garden, pH 7·2, a black garden loam.
Gault, pH 7·65, a clay soil from over the gault horizon.
Ramsgate, pH 8·0, a light chalk soil from Kent.

Table VII

Growth of fungus along the roots

	Richmond	Welsh	Devon	Slough	Chelsea	Gault	Ramsgate	Sand
pH value:								
Steamed	4·4	4·7	4·85	6·75	7·3	7·75	8·0	6·6
Not steamed	4·8	4·8	4·9	6·8	7·2	7·65	8·0	6·6
Growth of fungus in mm.:								
Steamed	0	2	4	22	25	21	25	28
Not steamed	8	13	11	13	20	25	28	28
Standard errors:								
Steamed	—	±0·7	±0·6	±1·2	±1·4	±0·8	±1·4	±0·9
Not steamed	±1·1	±0·7	±1·0	±0·6	±0·9	±1·0	±1·3	±0·9

In this experiment (Table VII) the soils fall into the same order for growth of the fungus as for pH value. Steaming brings about some improvement in the two more or less neutral soils, has a scarcely significant effect on the two alkaline soils, and a markedly adverse effect on the three acid soils. It might have been expected in this and in the following experiment that the effect of soil texture, particularly as influencing soil aeration, would have been more pronounced. But the close and uniform packing of the soil in the tumblers, and its high moisture content, are such as to minimize the effect of physical variations from one soil to another and to throw emphasis upon chemical factors, such as that of pH value.

Another experiment of the same kind, again with seven soils of which two, Slough and Chelsea, were from the same locations as in the experiment above, may be given here (Table VIII). The soils were from air-dried stocks, and were filled into the tumblers at 70 per cent saturation exactly as in the preceding experiment. The seven soils were as follows:

Jersey St Mary, *p*H 5·0, a "mid-season" potato soil.
Slough, *p*H 6·7, as in experiment above.
Spalding, *p*H 6·9, a light loam from Lincolnshire.
Chelsea Physic Garden, *p*H 6·95, as in experiment above.
Jersey L'Etacq, *p*H 6·95, an "early" potato soil, very light and sandy.
Skegness, *p*H 7·0, similar to Spalding soil.
Rothamsted, *p*H 7·5, a clay soil.

Table VIII

Growth of fungus along the roots

	Jersey St Mary	Slough	Spalding	Chelsea	Jersey L'Etacq	Skegness	Rothamsted	Sand
*p*H value	5·0	6·7	6·9	6·95	6·95	7·0	7·5	7·1
Growth of fungus in mm.	4	9	17	18	23	19	33	49
Standard errors	±0·6	±1·1	±1·2	±0·9	±1·3	±1·9	±2·6	±1·8

Here again the soils fall into the same order for growth of the fungus along the roots as that for *p*H value, with but one exception, that of Jersey L'Etacq. But this soil is exceptionally light and sandy, and the higher figure agrees well with expectation from its open and porous nature.

The effect of soil *p*H value upon the rate of growth of *Ophiobolus* along the roots was so strongly brought out by the foregoing experiments that a further investigation into the relation of *p*H value to the growth of the fungus seemed desirable. The fungus was therefore grown on the surface of sterilized Slough soil of different (adjusted) *p*H values in Petri dishes, and the growth measured after 10 days at 20° C. (Table IX).

Table IX

Growth of fungus on surface of sterile soil

	H_2SO_4				Un-treated	NaOH		
	0·4 %	0·3 %	0·2 %	0·1 %		0·05 %	0·10 %	0·15 %
*p*H value	6·1	6·55	6·7	6·9	7·15	7·25	7·35	7·5
Growth of fungus in mm.	30	33	33	33	34	33	34	34
Standard errors	±0·7	±1·0	±1·4	±0·4	±0·6	±1·7	±0·4	±0·4

Growing on the surface of sterilized soil, therefore, *Ophiobolus* is indifferent to its reaction over the range *p*H 6·5–7·5 investigated. This somewhat unexpected result was confirmed by another experiment, in which, however, 0·5 g. glucose per 100 g. of dry soil was added to encourage a denser growth of the fungus. It will be observed in Table X that one effect of the addition of glucose is a lowering of *p*H value relative to those of the same soils without glucose, as given in Table IX.

Table X

Growth of fungus on surface of sterile soil + 0·5 *per cent glucose*

	H_2SO_4			NaOH		
	0·4 %	0·2 %	Un-treated	0·1 %	0·2 %	0·3 %
*p*H value	5·7	6·0	6·4	6·7	7·0	7·3
Growth of fungus in mm.	25	24	31	28	29	31
Standard errors	±1·4	±0·6	±0·7	±1·3	±0·8	±1·0

Whilst in this experiment there is a significant decrease of growth on the soil at *p*H 6·0, once again, however, there is no significant difference in growth over the range *p*H 6·4–7·3. This conclusion is in general agreement with that of Webb & Fellows (60), who investigated the growth of *Ophiobolus graminis* at different *p*H values on five culture media. They concluded that "In general *O. graminis* grew well in culture over a wide range of active acidity and alkalinity. While the hydrogen ion generally proved more toxic than the hydroxyl ion, the toxicity of each was variable, depending upon the medium. It was only in a potato-dextrose decoction that the fungus was particularly sensitive to a condition of active alkalinity." It is therefore concluded that alkaline soils are no more favourable to the fungus as a nutritive substrate than neutral or slightly acid soils. The better growth of the fungus along the roots under the former condition must therefore be related to some factor other than the nutritive one.

Briefly summarizing the results of the foregoing experiments, it may be said that whilst the fungus grows along the roots best of all in sand, its growth in soil is accelerated by any measures which promote better soil aeration. In the second place, the fungus growing along the roots is markedly susceptible to the *p*H value of the soil, and its growth rate increases in a regular manner with the transition from acid to alkaline soils. It has also been shown, however, that alkaline soils are no more favourable to the fungus as a nutritive substrate than neutral or slightly acid soils. The effect of steaming a soil upon the rate of growth of the fungus is somewhat variable; in the case of neutral and alkaline soils, a marked improvement is effected, but in the case of the more acid soils the effect is a decidedly adverse one.

(*b*) *Partial sterilization of the soil in relation to growth of the fungus along the roots*

The partial sterilization of soils is well known to result in profound microbiological changes. Beginning with the work of Russell and his associates, this problem has received attention from many investigators; their results have been reviewed and discussed by Waksman & Starkey (59).

In the earlier stages of this work (17) it was considered that the more rapid growth of the fungus in a steam-sterilized soil was due to the sterilization effect. Further experiments have shown, however, that such an improvement is dependent upon the actual method of partial sterilization, and is therefore to be attributed to some chemical or physical change rather than to the biological one. In one such experiment, Slough soil was sterilized in four different ways, by steam, formaldehyde, alcohol and toluol. The steam-sterilized soil was autoclaved for 2½ hours at 15 lb. pressure. The formaldehyde treated soil was made up to 50 per cent saturation with 1 per cent formaldehyde, and kept in a covered dish for 24 hours. The toluol-treated soil received the maximum dose employed by Buddin (7) in his work on the partial sterilization of soil by chemicals; this maximum dose was fixed as one molecular weight of the chemical to 1 kg. of dry soil. Accordingly, toluol was added to the moist soil (30 per cent saturation) at the rate of 90 g. of toluol to 1 kg. of the dry soil, and the treated soil kept in a covered dish for 24 hours. The alcohol-treated soil was made up to 50 per cent saturation with 50 per cent alcohol, and kept in a covered dish for 24 hours.

After these treatments, the soils were spread out for evaporation of the volatile chemicals, and kept for 10 days. Tumblers were then filled with the soils at 70 per cent saturation in the usual manner, and the growth of the fungus along the roots in the different soils was measured (Table XI).

Table XI

Growth of fungus along the roots

	Untreated	Alcohol	Toluol	Form-aldehyde	Steam
Growth of fungus in mm.	11	12	15	24	23
Standard errors	±1·0	±0·8	±1·0	±1·1	±1·0

It will be observed that whilst both the steam and the formaldehyde treatments have more than doubled the growth of the fungus, the alcohol treatment has produced no significant effect upon the soil, and the difference between the toluol treated soil and the control is only just significant (4 ± 1·4). All these treatments must have produced substantially the same microbiological effect, in killing all but the most resistant organisms. It therefore follows that the favourable effect of the steam and formaldehyde treatments cannot be due to the sterilizing action of the two treatments, but must be attributed to some chemical or physical effect.

Furthermore, experiments have shown that a totally sterilized soil reinoculated with its own microflora is at first no less favourable to the growth of *Ophiobolus* than one kept absolutely sterile. In one such experiment, a dozen tumblers were filled with Slough soil in the usual manner and autoclaved for 3 hours at 15 lb. pressure. After cooling, the final 15 c.c. of water was added to each tumbler, together with the weight of water lost in the autoclave. To six of the tumblers this addition was made as sterile water, and the tumblers were inoculated, planted and incubated under sterile conditions. To the other six the final addition of water was made in the form of a suspension of unsterilized soil, obtained by shaking 100 g. of soil up with 1000 c.c. of water. The tumblers were then seeded (Table XII).

Table XII

Growth of fungus along the roots

	Untreated	Kept sterile	Reinoculated
Growth of fungus in m	12	24	23
Standard errors	±0·7	±1·0	±1·1
Bacterial numbers in millions per g. of air-dry soil	30	0	110

In spite of the fact that bacterial numbers, determined at the end of the experiment by plate count on Thornton's medium, were considerably higher in the reinoculated soil than in the untreated control soil, the reinoculation has had no significant effect upon the growth of *Ophiobolus* along the roots during the period of the experiment (16 days).

Under the closely packed condition of the soil in the tumblers, it is only after the reinoculated soil has been incubated for a period of some 3 weeks at a temperature of 25° C. that a deterioration may be perceptible. If, however, the soil be incubated at 50 per cent saturation and loosely packed so as to favour aeration, then deterioration, i.e. return to normal, is much more rapid. Thus in a typical experiment, Slough soil previously steamed at atmospheric pressure (i.e. partially sterilized only) and then air dried was incubated for 3 weeks at 25° C. (i) at 50 per cent saturation, loosely packed, (ii) at 70 per cent saturation, closely packed, exactly as in the tumblers. At the end of this incubation period, both soils were air dried, and then filled into the tumblers at 70 per cent saturation in the usual manner, along with a control soil, not steamed, and another control, steamed but not incubated. The pH of all soils was found to be 6·8 (Table XIII).

Table XIII

Growth of fungus along the roots.

	Steamed soils			Not steamed
		Incubated		
	No incubation	At 70 % saturation	At 50 % saturation	Control
Growth of fungus in mm.	30	24	17	12
Standard errors	±1·0	±1·1	±1·1	±1·0

All the differences in Table XIII are significant; it will be seen that the return of the steamed soil towards normal is much more rapid when the soil is incubated in the loosely packed condition at 50 per cent saturation. If this return of the steamed soil towards normal is the result of microbiological action, then the more rapid change at 50 per cent saturation might be due to (i) development of greater numbers of organisms, (ii) greater individual activity of organisms, (iii) a qualitative difference in the soil microflora developing under the condition of better soil aeration. That such a qualitative difference did in fact occur was indicated by a profuse visible development of fungus mycelium appearing in the soil at 50 per cent saturation, but not in that at 70 per cent saturation.

In another experiment, in which the steamed soil was made alkaline by addition of 0·15 per cent of sodium hydroxide before incubation, a similar result was obtained (Table XIV).

Table XIV

Growth of fungus along the roots

		Incubated	
	No. incubation	At 70 % saturation	At 50 % saturation
Soil pH	8·2	8·2	8·05
Growth of fungus in mm.	28	24	14
Standard errors	±2·7	±1·4	±1·1

The difference between the soil incubated at 70 per cent saturation and that having no incubation is not significant. But growth made in the soil incubated at 50 per cent saturation is only one-half that made in the non-incubated control in spite of the fact that the difference of pH value amounts to only 0·15 of a unit. It is thus possible for two soils of almost the same pH value to vary considerably as an environment for the fungus. In Table V it can be seen that growth in a steamed soil of pH 7·5 is 37 mm., as against a growth of only 26 mm. in an unsteamed soil of corresponding reaction pH 7·6. In that experiment steaming produced a considerable improvement in the soil as an environment for the fungus, irrespective of any change in pH value; in this experiment exactly the converse has occurred, viz. the reversion of the treated soil towards normal, again without material change of pH. It thus appears that the pH value of the soil is not the only chemical factor influencing the rate of growth of the fungus.

Summarizing the results of the foregoing experiments it appears that whilst the return of a steamed or alkali-treated soil towards normal is presumably the result of microbiological action, and varies in rate with the conditions of incubation of the soil, the original improvement brought about by steaming must be of a chemical or physical but not biological nature.

IV. DISCUSSION

(a) *Interpretation of the experimental work*

As a result of the experimental work set out in the preceding section, it has been found that *Ophiobolus graminis* grows along the roots of wheat plants best of all in sand, and that its growth in soil is favoured by any condition tending to promote better aeration, by rise in pH value of the soil, and by steaming, except in the case of the more acid soils. It has been shown, furthermore, that the improvement brought about by steaming cannot be attributed to the effect of partial sterilization as such, since it is not produced by partial sterilization with alcohol or with toluol, but must be ascribed to some chemical or physical change produced in the soil by the action of steaming. Formaldehyde has also been found to effect such an improvement in the soil.

Some attempt must now be made to incorporate these facts into a general hypothesis whereby the effect of soil conditions upon the rate of growth of *Ophiobolus* along the roots can be explained and predicted. In the first place, it may be suggested that the observed variations in growth of the fungus are due in part, if not entirely, to variations in resistance of the wheat roots under different soil conditions. This suggestion is, however, discounted by an experiment already published (17), in which wheat plants were grown in a pure quartz sand under four very different conditions of nutrition and illumination: (*a*) in full sunlight with a full nutrient solution, (*b*) in full sunlight without nutrient solution, (*c*) in poor diffuse light with full nutrient solution, (*d*) in poor diffuse light without nutrient solution. The plants were grown under these conditions for 16 days, by which time striking differences in appearance were manifest, and were then inoculated with the fungus. No significant differences in the rate of growth of *Ophiobolus* along the roots of these very differently treated plants could be detected. It is therefore concluded that root resistance is not a factor likely to have influenced the rate of growth of the fungus along the roots in these experiments.

In the earlier work on this problem (17), the poor growth of *Ophiobolus* along the roots in the heavier soils was attributed to the operation of a microbiological factor, viz. the antagonism of the soil micro-organisms, which was reasonably assumed to be greater in the heavier soils with greater organic content. This hypothesis was supported by experiments in which it was found that such soils became much more favourable to the growth of the fungus when steam sterilized. The experiments quoted above have shown, however, that this improvement is independent of the biological factor, and must be attributed to some chemical or physical change brought about by the steaming. Microbiological antagonism is probably of considerable importance in relation to the saprophytic decomposition of *Ophiobolus* mycelium in the resting stage, and must vary according to the soil conditions; the evidence of the experiments here reported indicates, however, that it does not directly affect the rate of growth of the fungus along the roots, except in so far as it gradually brings about general physical and chemical changes in the soil, as in the return of a steamed soil to normal.

The fact that the fungus grows along the roots best of all in pure sand suggests that its growth in soil is governed by a retarding factor, and that variations in the growth of the fungus are to be related to variations in the degree of its incidence. It is not easy, however, to postulate a retarding factor which will fit in with all the facts. It is not present in sand; in soil it is neutralized by alkalinity, but enhanced by acidity. Moreover, whilst it is to some extent removed by steam treatment in neutral soils, in the more acid soils it is actually increased; i.e. it is thermolabile at one pH and thermostable at another. Nor can the better growth of the fungus along the roots in alkaline soils be attributed to any preference of the fungus for alkaline soils as a nutritive substrate, for this possibility has been ruled out by experiment.

It is as idle as it is unnecessary to postulate a complex retarding factor (22), and such a possibility has already been to some extent discounted by the work of Hutchinson & Thaysen (27). Whilst, under conditions of pure culture on artificial media, it is possible to conceive of almost any of the products of growth as able at a sufficient concentration to stop the advance of a fungus, in the soil the by-products of one organism form the substrate of another. When it is considered that even toxic chemicals like toluene, carbon disulphide and naphthalene have been shown (7, 28, 34) to be decomposed quite rapidly in the soil, it is hard to imagine that any complex substance retarding the growth of *Ophiobolus* can accumulate in the soil. It is possible, on the other hand, to conceive of the temporary accumulation of carbon dioxide, which is the final product of combustion.

Consideration of the fact that *Ophiobolus* makes the best growth of all, on the one hand in sand and on the other in alkaline soils, has led to the realization that carbon dioxide may indeed be this retarding factor. This possibility has already been examined by Fellows (13), who found that the growth rate of the fungus on potato-dextrose agar fell off with increasing atmospheric concentrations of the gas. Fellows concludes, however, that "the variations in carbon dioxide and oxygen as found in arable soils are not great enough to affect materially the growth of *Ophiobolus graminis*", in view of the fact that the carbon dioxide concentration in the general soil atmosphere, under normal conditions, seldom reaches even 1 per cent (47). Such determinations of the average concentration of carbon dioxide in the soil atmosphere are of value, but, at the same time, they can give no indication of the wide variations in local concentration that must occur. Thus the zone in which the fungus hyphae are actually growing, i.e. that between the root and the soil, must have a local or microclimate (2) in which the carbon dioxide percentage may be considerably higher, owing to the respiration of the plant root, and therefore probably quite sufficient to affect the growth of the fungus. The actual concentration of carbon dioxide in this microclimate must depend upon (i) its rate of production by the respiration of the plant root, the hyphae of *Ophiobolus*, and the general soil microflora, (ii) upon the closeness with which the soil invests the root, (iii) upon the physical nature of the soil, determining the rate of diffusion of the gas away from the root, (iv) upon the chemical nature of the soil. Alkaline soils should act as carbon dioxide acceptors, rapidly reducing the concentration of carbon dioxide in the root zone.

It is suggested, therefore, that sand and sandy soils are most favourable to the growth of the fungus along the roots in virtue of the more rapid physical diffusion of carbon dioxide away from the root under such conditions, and that alkaline soils are congenial in that they may act as carbon dioxide acceptors to the root zone. The power of an alkaline soil to take up carbon dioxide as bicarbonate should increase directly with the pH value of the soil, since the equilibrium between free carbon dioxide and bicarbonate is determined by pH, thus (8):

$$\frac{[\mathrm{HCO_3^-}]}{[\text{free } \mathrm{CO_2}]}=\frac{K_1'}{[\mathrm{H^+}]}, \qquad \ldots\ldots(a)$$

where K_1' is a constant and H^+ represents the hydrogen-ion concentration. The ratio between free carbon dioxide and bicarbonate at any pH may be easily worked out in percentage from the following relation:

$$p\mathrm{H}=pK_1'+\log\frac{[\mathrm{HCO_3^-}]}{[\text{free } \mathrm{CO_2}]}, \qquad \ldots\ldots(b)$$

where K_1' is the same constant as that of equation (a), and pK_1' represents the pH value at which [free CO_2] is equal to [HCO_3^-]. Taking this value as 6·45, the relation between free carbon dioxide and bicarbonate has been worked out for pH values from 5·5 to 8·0, and may be seen in Table XV.

Table XV

Relation between free carbon dioxide and bicarbonate at different pH *values*

pH	% CO_2	% HCO^-
5·5	90	10
6·0	74	26
6·45	50	50
7·0	22	78
7·5	8	92
8·0	3	97

The power of the soil to take up carbon dioxide as bicarbonate from the root zone should thus increase with rise in pH value from pH 5·5 upwards. This hypothesis does not, of course, necessarily imply any appreciable concentration of bicarbonate around the root, since any such accumulation would at once upset the equilibrium between carbonate and bicarbonate in the general soil solution, and restoration of this equilibrium would be very rapid. In an acid soil, on the other hand, diffusion of carbon dioxide away from the root must be almost entirely physical, and hence very slow indeed in a soil at 70 per cent saturation. Under such conditions, the rate of diffusion might be expected to approximate to that in absolutely still water.

Whilst the existence of an alkaline soil reaction is thus held to be favourable to the fungus in virtue of the low concentration of free carbon dioxide which it entails, it must be remembered that the bicarbonate ion can itself be an active inhibitor of fungal growth. Thus Pratt (42) has shown that the factor responsible for the staling of a number of fungi is the accumulation of bicarbonate when the medium is such that it becomes alkaline through fungal growth. The concentration of bicarbonate found by Pratt in stale culture media corresponded to 650 parts per million of carbon dioxide; only under rather exceptional conditions, however, is such a high concentration of bicarbonate found to occur in the soil solution.

As an illustration of the manner in which the power of the soil to act as a CO_2 acceptor varies with the *p*H value, the following experiment may be cited. Two series of tumblers were filled with Slough soil at 70 per cent saturation in the usual manner, but in the place of water dilute sulphuric acid and dilute sodium hydroxide, respectively, were employed, in such strengths that the resulting soils contained 0·3 per cent sulphuric acid and 0·15 per cent sodium hydroxide. A third series was prepared using water alone. The tumblers were placed in the 20° C. incubator.

After 1 week, determinations of the power of the different soils to take up carbon dioxide were made as follows. A tumbler was fitted with a vaselined rubber bung fitting right down to soil level. It was then inverted on top of a large Haldane's gas analysis burette and a direct connexion made through one limb of a two-way cock. The other limb was in turn connected with (1) a filter pump, whereby the tumbler was evacuated for 5 min., (2) a reservoir of air containing 2 per cent carbon dioxide for 5 min. Connexion was then cut off, and two successive samples of 6 c.c. of soil air withdrawn from the tumbler for analysis in the gas burette. The percentage of carbon dioxide taken up by the soil from that admitted from the reservoir could then be calculated. Three determinations, each in duplicate, were made for each of the three soil series, at weekly intervals (Table XVI).

Table XVI

Percentage CO_2 *taken up by soil from* CO_2 *passed in*

	0·3 % H_2SO_4	Untreated	0·15 % NaOH
After 1 week	18	58	77
After 2 weeks	36	61	71
After 3 weeks	48	61	80

It will be observed that whilst treatment with alkali enhances the power of the soil to take up carbon dioxide, acid treatment depresses it, but in the case of the acidified soil there appears to be a gradual recovery over the period of 3 weeks in which determinations were carried out.

An essential postulate of the hypothesis presented above is that the fungus be sensitive to concentrations of carbon dioxide likely to occur in the microclimate of the root zone. The growth of the fungus at different atmospheric concentrations of carbon dioxide was therefore studied, using the technique devised by Brown (6). The different experimental atmospheres, 0, 5, 10, 15 and 20 per cent carbon dioxide, respectively, were set up in large vessels, of the type used for desiccators, of some 9 litres capacity, and checked by analysis with the Haldane apparatus. Eight plate cultures of the fungus were grown at each concentration of carbon dioxide; potato-dextrose agar was used and the plates poured rather deep to prevent staling, as recommended by Brown. To facilitate gaseous diffusion, the lids of the Petri dishes were removed and the dishes strapped together in bundles of four, upside down, with a clean sterile dish at the bottom of each pile. The experiment was made at laboratory temperature, which was maintained at 21–23° C. throughout. After 6 days, diameters of the fungus colonies were measured, and the growth made in different concentrations of carbon dioxide calculated (Table XVII).

Table XVII

Growth of the fungus on potato-dextrose agar at different atmospheric concentrations of carbon dioxide

Percentage CO_2	0	5	10	15	20
Growth of fungus in mm.	27	20	18·5	8·5	11
Standard errors	±0·3	±1·2	±0·6	±2·1	±2·2

In an atmosphere of 15 per cent carbon dioxide, the growth of the fungus is reduced to less than 30 per cent of its value in air. Growing on potato-dextrose agar, therefore, *Ophiobolus graminis* appears to be sufficiently sensitive to the atmospheric concentration of carbon dioxide to justify the hypothesis put forward above. Growing upon the wheat root, the fungus may be more sensitive still, for Brown (6) concluded, as a result of his work with a number of different fungi, that the retarding effect of the carbon dioxide factor varied with the nature of the substrate and with the other conditions of the environment; in general, the retardation was greatest when the energy of growth of the fungus was least, e.g. on a poor substrate and at a temperature unfavourable for growth. Whilst the wheat root is indeed the natural substrate of *Ophiobolus*, it is yet not so favourable for growth as potato-dextrose agar. For even under the best conditions the growth rate of the fungus along the roots is never appreciably more than half that on potato-dextrose agar.

It is obviously a matter of extreme experimental difficulty to obtain direct evidence of the concentration of carbon dioxide in the atmosphere immediately in contact with the root. The sampling would have to be done with the root growing *in situ* in the soil; at the same time, the very act of sampling would upset the process of diffusion and the carbon dioxide/bicarbonate equilibrium upon which the concentration of carbon dioxide in this zone must depend. The rate of production of carbon dioxide by the roots of various crop plants has been studied by Newton (38). A very rough approximation from his data suggests that a wheat root may respire its own volume of carbon dioxide in 24 hours. The respiration of the *Ophiobolus* hyphae must contribute something to the total output of an infected root, which probably respires more actively than a healthy one (30). The respiration of the general soil microflora around the root must also be taken into account. The possible significance of this contribution is strongly emphasized by the work of Starkey (57), who found that the numbers and activity of the soil micro-organisms increased greatly in the root zone; on an average of all the plants investigated, the number of bacteria per g. of soil at the root surface was some 25 times as great as that in the soil close to the roots.

Whilst the respiration of the general soil microflora may thus contribute directly to the production of carbon dioxide in the root zone, it must at the same time have an indirect effect more important still. Granted a type of soil which acts as a carbon dioxide acceptor, the growth of micro-organisms would tend to eliminate this character. Thus the improvement effected by a treatment with alkali has been found by experiment to fall off with lapse of time as a result of microbiological action. This may be attributed in part to changes produced by the growth and assimilation of the organisms in the soil bases which act as carbon dioxide acceptors, and in part to the accumulation of respiratory carbon dioxide in the incubated soil. For the rate of diffusion of carbon dioxide from the root zone will be much less rapid towards a sink of 1 per cent carbon dioxide than towards a sink of zero concentration. In maintaining a general concentration of the order of 1 per cent carbon dioxide in the soil atmosphere, therefore, the respiration of the soil microflora may result in a much larger increase in the concentration at the root zone.

In conclusion, the case of steamed and formaldehyde-treated soils must be discussed in relation to the hypothesis presented above. The experimental evidence has shown that the improvement effected by these two treatments can only be attributed to the production of some chemical or physical change. The effect of formaldehyde must remain open to investigation. The interpretation of the favourable effect of steaming on the growth of the fungus, however, offers no difficulties. Although the changes produced in the soil by steaming are incompletely understood, there is no doubt that at least in soils not too far removed from neutrality, steaming accentuates the power of the soil to act as a carbon dioxide acceptor. Free carbon dioxide is driven off, and bicarbonates are changed into carbonates. Moreover, there is an appreciable amount of alkaline ammonium carbonate formed in steamed soils—so much so that the toxicity of certain steamed soils to seedling growth has been attributed to this cause by Johnson (29). It is certainly suggestive that the more acid soils, which can have little power of taking up carbon dioxide, are not improved by steam treatment.

The carbon dioxide hypothesis, while accounting reasonably well for all the observed facts, must still be regarded as non-proven. It is easy to suggest diverse alternative hypotheses; soil reaction, whilst not directly influencing the fungus, might yet influence its parasitism of the root, either through a neutralization of the stimulus to growth along the root (root excretions?), or through some direct effect upon the (enzymic?) mechanism of penetration. Such alternative hypotheses all involve additional assumptions, however, and are hence inferior to the carbon dioxide hypothesis, which has at least the merit of simplicity.

(*b*) *Relation of the experimental results to the occurrence of the take-all disease in the field*

It has appeared from these experiments that growth of *Ophiobolus graminis* along the roots is most rapid under just those soil conditions which are known to favour the incidence of the take-all disease in the field, viz. loose and open soils, soils of light texture, and alkaline soils. This is not at all surprising, however, since the rate of growth of the fungus along the root system must be one of the chief factors determining whether the attack be fatal to the plant or not. The speed with which the fungus reaches the crown region from one or more foci of infection on outlying parts of the root system may be the decisive factor in the recovery or otherwise of the plant. Once the fungus has established itself around the crown, new secondary roots may be destroyed almost at their inception. Of considerable interest in this connexion are the recent observations of Fellows & Ficke (16), who planted wheat at different levels above a layer of *Ophiobolus* inoculum in the soil, and found that the attack was only fatal when the inoculum was 3 in. or less below the seed.

Since it has been found in the present investigation that the fungus cannot extend, apparently, for more than a few millimetres if confined to the inside of the root, its dependence upon the soil environment must be a very real one.

Soil conditions are generally considered, however, to influence not only the fungal parasite but also the resistance of the host plant. Perhaps the best example of such an effect amongst the cereal foot-rot fungi is afforded by the work of J. G. Dickson (10) and his collaborators (11) on the seedling blights of wheat and maize due to *Gibberella saubinetii*. But in the case of *Ophiobolus graminis* the writer has failed to find any indication of resistance to the fungus in the seminal roots of wheat, either by experiment (17) or through general observation. This is supported by the work of Fellows (14) and Robertson (45). The importance of the seminal roots to the plant has, of late, been emphasized by the root-cutting studies of Simmonds & Sallans (55, 56), who conclude that during the seedling period and up to about mid-season, the seminal roots appear to be the most important and constitute the chief absorbing system. This is supported by the earlier work of Krassovsky (33) on water and salt absorption. The only investigations to have been reported, so far, on the resistance of the crown roots to *Ophiobolus* are the histological studies of Robertson (45). He finds that a marked increase in the amount of lignified tissue takes place in the crown roots with advancing age, reaching a maximum by about 40 days in pot culture. It is possible, therefore, that this lignification may confer some degree of resistance upon the plant. Experiments to test this were carried out by Broadfoot (5), but were unfortunately vitiated by another variable factor, viz. microbiological antagonism to the pathogen in the soil, which also increased with the age of the pot cultures. It is possible, again, that the power of an injured plant to put out new crown roots (55, 56) may also be a factor making for resistance under some conditions.

In conclusion, reference may be made to an observation, quite possibly typical of others not reported, which at first sight appears to conflict with the conclusions reported in this paper. Thus it has been recently reported by Åkerman *et al.* (1) that insufficiently rotted stable manure has been observed to stimulate the development of the take-all disease. It may here be argued, however, that the microbiological action of organic matter in controlling the disease was in this case more than offset by the harmful opening up of the soil through the undecomposed straw residues.

V. CONTROL

These investigations suggest that control of the take-all disease will be assisted by any measures which:

(1) Compact the soil. This fact is already well known to wheat farmers in South Australia (23).

(2) Reduce the open character of the more sandy soils, e.g. by increasing the humus content.

(3) Lower the pH value of the soil.

(4) Increase the activity of the soil microflora, e.g. by the incorporation of organic matter in the soil (15). If bulky plant residues slow of decomposition are employed, however, the soil may be opened up too much, and the reverse effect obtained (1).

On soils of light texture and alkaline reaction, such as the mallee areas of South Australia, these desirable changes can only be economically effected by a long-term agricultural policy designed to increase the humus content of the soil (17). By such a policy the loose and open character of the lighter soils will be appreciably modified, their alkalinity will be reduced, and the increase in organic content and microbiological activity should lead, on the one hand, to increased soil respiration, retarding the activity of *Ophiobolus* on the roots of the growing crop, and on the other to the more rapid decomposition of the resting mycelium in the fallow periods.

It is interesting to see that such a desirable change is indeed already progressing, though slowly, in the mallee soils of South Australia. With the progress of cultivation, greater compaction of the soil can be obtained, and at the same time the humus content gradually increases. Nevertheless, there can be no doubt that this evolution of the virgin mallee soil into one less congenial to take-all could still be considerably hastened by the increased use of green crops in the rotation, and especially by the feeding off of such crops with sheep, which are already kept by many of the wheat farmers.

Good support for this policy is afforded by a consideration of the prevalence of the disease on the mallee soils of the State (17). Whilst take-all is still very prevalent on areas cleared less than 20 years ago from virgin scrub, it is generally no longer a serious problem on soils some 25 years under cultivation, and on soils older still it has practically disappeared, under good farming practice.

Soil reaction appears to exercise such a marked effect upon the activity of the fungus that attention might well be given to special measures for lowering the pH value of the soil, where this is at all feasible. It seems just possible that the favourable effect of superphosphate in reducing the disease may be due in part to a temporary lowering of soil pH in the immediate neighbourhood of the seed and crown of the plant, which may be termed "a key position" for the fungus. It is possible, again, that the application of ammonium sulphate would be of some value in this respect. The control of take-all by ferrous sulphate has been reported by Darnell-Smith (9). The control of the disease by the application of sulphuric acid has long been practised in France.

Lastly, the realization that the fungus can increase in the soil only on living plants and must deteriorate in their absence through the antagonism of the soil saprophytes emphasizes once more the importance of grass hosts in the propagation of the disease (40, 41). In the South Australian mallee areas, a crop sown on land cultivated after a 2-year spell as pasture generally suffered severely from take-all. R. L. Griffiths (23) recommends for those areas the replacement of the highly susceptible and ubiquitous barley grass (*Hordeum murinum*) pasture by a seeded pasture of Wimmera rye-grass (*Lolium subulatum*), which is apparently immune to take-all, to be drilled in with the wheat.

Soil amendment alone, therefore, should not be regarded as a sovereign remedy for the take-all disease, but is rather to be adopted as an addition to those practices already in use for its control, viz. rotation of crops, early and well-worked fallows, eradication of susceptible grasses, burning of the stubble, and proper compaction of the soil and preparation of the seed-bed. Indeed, the neglect of these precautions may lead to a severe outbreak of take-all even on soils where it is seldom seen.

VI. SUMMARY

Ophiobolus graminis can spread through the soil only along the roots of its host plants. A distinction can thus be made between two phases in the activity of the fungus, a parasitic or ascendant phase, in which the fungus is actively increasing on the roots, and a pseudo-saprophytic or declining phase, in which the fungus is merely persisting in dead host tissue. In the declining phase, the disappearance of the fungus from the soil must be hastened by the action of the soil saprophytes in actually decomposing its mycelium. Certain soil conditions, which at one time increase the activity of the fungus on the roots of its host, may at another time hasten the disappearance of the resting mycelium from a fallow soil.

The rate of growth of *Ophiobolus graminis* along the roots of wheat seedlings has been found to vary widely with soil conditions, and growth may even be inhibited altogether. The best growth of all is made in sand; growth in soil increases both with improvement in aeration, and with rise in pH value; it is thus best in light and open soils, and in alkaline soils. In the case of neutral and alkaline soils, growth may be improved by steaming.

A hypothesis has been put forward whereby the rate of growth of the fungus along the roots is related to the concentration of carbon dioxide in the microclimate of the root zone, along which the fungal hyphae are growing. Carbon dioxide is produced by the respiration of the host root, the hyphae of *Ophiobolus*, and the soil microflora; variation in the concentration of carbon dioxide in the root zone will depend on the rate of diffusion of the gas away from the root. This will be most rapid, on the one hand, in light and open soils favouring rapid physical diffusion, and, on the other, in alkaline soils, which can act chemically as carbon dioxide acceptors. These are just the soil conditions which make for most rapid growth of the fungus along the roots.

Growth of *Ophiobolus graminis* along the roots is also most rapid under just those soil conditions known to favour the occurrence of the take-all disease in the field, viz. loose and open soils, soils of light texture, and alkaline soils. This well-established action of soil conditions upon the prevalence of the disease in the field is thus considered to operate directly through the fungus, rather than indirectly through the resistance, yet to be demonstrated, of the host plant.

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A DISEASE OF CHERRY LAUREL CAUSED BY *TROCHILA LAUROCERASI* (DESM.) FR.

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(With Plate XXXI)

Introduction

Trochila Laurocerasi (Desm.) Fr. has long been known as a saprophyte growing on dead leaves of the cherry laurel (*Prunus Laurocerasus*). In the spring of 1932, however, the writer received some living leaves of cherry laurel bearing large irregular brown spots upon which had developed apothecia of *Trochila Laurocerasi* and also numerous acervuli of a *Gloeosporium* species (Pl. XXXI, fig. 1). The specimens came from a fine cherry laurel hedge in Dumfriesshire which was said to be almost ruined as a result of the disease. They had been sent first to the Ministry of Agriculture's Plant Pathological Laboratory at Harpenden, and the fungus had been identified by Miss E. M. Wakefield of Kew. She stated that she had seen a similar case some years previously at Norwich, and had also on that occasion found indications of a possible conidial stage of the *Trochila*, but she had been unable to devote further time to the problem. Some of the Dumfriesshire specimens were forwarded subsequently to the Plant Pathological Laboratory of the Department of Agriculture for Scotland with the suggestion that the matter would repay closer study.

Description of the disease

The earliest external symptom is the development on the leaves of yellowish spots with a very indefinite margin. Later these become sharply delimited by the formation of a thickened ring of tissue separating the discoloured area from the healthy portion of the leaf. Sometimes this ring is not developed rapidly enough to check the advance of the fungus and the discoloration then extends beyond it and another ring is formed in the path of the parasite (Pl. XXXI, fig. 2). It is not uncommon to find several such rings, more or less concentrically arranged, upon the surface of the larger spots. Meanwhile the infected areas gradually change colour, commencing at the centre. They first assume a purplish tinge and later become brown, the shade varying from greyish brown to a deep red brown, sometimes being markedly different in adjacent rings of the same spot. Only after this last phase has been attained do the fructifications of the fungus develop. They are mainly confined to the upper surface of the leaf but may occasionally develop also on the lower surface. *Gloeosporium* acervuli are formed first but are succeeded by the apothecia of *Trochila Laurocerasi*. In the later stages of the disease the discoloured spots become separated from the leaf along the inner side of the thickened marginal ring (Pl. XXXI, fig. 1); they then fall away, leaving large holes with thickened rims. These holes are not always spherical but may be irregular in outline and are sometimes cut out from the edge of the leaf. The method by which the diseased area becomes separated from the healthy tissues of the leaf is exactly similar to that described and illustrated by Blackman & Matthaei(1) and Samuel(3) in the case of mechanical injury to cherry laurel leaves.

Dr Pethybridge stated in correspondence that he had observed lesions on the green parts of the twigs as well as on the leaves, but these were not present on the material examined by the present writer.

CULTURE STUDIES

The ascospores of *Trochila Laurocerasi* germinate readily within 24 hours in a hanging drop of sterile water. Each spore is unicellular, contains very granular protoplasm and is surrounded by a thick sheath of mucilage. One or more germ tubes may be formed from any part of the spore but most commonly from one or both ends. Anastomoses may occur between germ tubes from adjoining spores or between a germ tube and a neighbouring spore. After 48 hours many of the germinating spores, and occasionally some which have failed to germinate, become divided by a transverse septum.

The average diameter of a monospore culture on malt-extract agar after 7 days at room temperature is 0·7 cm.: at this stage it is pure white with well-developed fluffy aerial mycelium. After about 14 days a flatter marginal zone becomes differentiated on the lower side of the growth in a tube culture; this gradually assumes a darker colour and drops of moisture are exuded from the mycelium. Ultimately this lower part becomes brownish black with a wrinkled slimy surface due to the production of vast numbers of *Gloeosporium* conidia. These germinate in a manner similar to that described for the ascospores, and, like the latter, they frequently become divided by a transverse septum. Single conidia sown on malt-extract agar give rise to cultures identical in every respect with those obtained from single ascospores.

Apothecia of *Trochila* never developed in agar cultures, but when pieces of sterilized cherry laurel leaves were employed as the nutrient medium, the *Gloeosporium* stage was rapidly succeeded by fructifications of *Trochila*. Mature ascospores were formed in about 14 days from the time of planting a single spore, and identical results were obtained whether the inoculum consisted of an ascospore or a conidium. There remains, therefore, no doubt that this *Gloeosporium* is the imperfect stage of *Trochila Laurocerasi*.

IDENTITY OF THE IMPERFECT STAGE

The conidial stage has been identified as *Gloeosporium phacidiellum* Grove, which was first described in 1912 (2). At the end of the description Grove added, "Presumably the pycnidium stage of *Trochila Laurocerasi*", but he did not test his hypothesis by cultural studies. He kindly lent his type specimen to the present writer, and it was found to agree in every detail with the living material. In his published description Grove gave the dimensions of the conidia as $18–20 \times 7–8\mu$. The measurements made by the present writer from 100 conidia taken at random from each specimen were: type specimen $12·3–17·9 \times 3·9–7·4\mu$, mean $15·0 \pm 0·11 \times 5·4 \pm 0·06\mu$; living material $11·9–18·6 \times 4·2–6·3\mu$, mean $14·9 \pm 0·12 \times 5·0 \pm 0·05\mu$. Thus it will be seen that the two specimens were practically identical with regard to size of conidia, but they differed decidedly in this respect from the published description. Grove, in correspondence, explained this apparent discrepancy by the fact that he did not measure any conidium which he thought might be immature. The present writer has, however, germinated many hundreds of conidia during this work, and it was found that the smallest were as capable of germination as the larger ones. Thus the series of measurements which includes these smaller conidia should probably be regarded as a more accurate expression of the degree of variability exhibited by this species in the matter of conidial dimensions.

INFECTION EXPERIMENTS

Numerous inoculations with monospore cultures of *Trochila Laurocerasi* were carried out during the spring and summer months of two successive years. Unusual difficulty was incurred owing to the fact, already mentioned, that the cherry laurel rapidly occludes any portions of its leaves which have been mechanically injured. A number of preliminary experiments was therefore carried out in order to ascertain whether any slight wound could be inflicted without bringing about this undesired effect. It was found that no isolation of the injured area took place if the epidermis was slit with a sharp razor, but if the cut penetrated deeply the usual callus was formed. All other types of injury led to the development of a callus and the subsequent separation of the occluded area.

The infection experiments were performed on small plants in pots. Before inoculation these were sprayed with sterile water from an atomizer, and for several days they were either covered by bell-jars or the inoculated parts were enclosed in cellophane cylinders plugged at both ends with cotton wool. No wound other than a slit in the epidermis was inflicted upon the leaves. Mycelium for inoculation was taken from the edges of young cultures, together with a fragment of the underlying agar. When spores were used as the inoculum two different methods were employed. In the first, portions of leaf culture bearing mature acervuli or apothecia were laid in contact with the living tissues for 24 hours. The second method consisted of painting a suspension of spores in sterile water on to the required spot with a sterilized brush, or, in the case of a few young stems, injecting the suspension with a hypodermic syringe. The results of the experiments are summarized in Table I. Control plants were kept but in no case did these become infected.

Table I

Infection experiments with Trochila Laurocerasi *on cherry laurel*

Part of plant inoculated	Whether wounded or unwounded	Inoculum	No. of inoculations	No. of infections
Leaves	Wounded	Mycelium	28	22
Leaves	Unwounded	Mycelium	22	3
Leaves	Wounded	Conidia	37	4
Leaves	Unwounded	Conidia	12	0
Leaves	Wounded	Ascospores	25	4
Leaves	Unwounded	Ascospores	33	0
Young stems	Wounded	Mycelium	7	0
Young stems	Unwounded	Mycelium	6	1
Young stems	Wounded	Conidia	18	0
Young stems	Unwounded	Conidia	10	0

Only young leaves contracted the disease. Attempts to infect leaves during late autumn or in their second year of growth yielded negative results, even when they were wounded before inoculation. Infection occurred as readily through the upper as through the lower surface of the leaf. It will be seen from Table I that infection of young unwounded leaves did occur, but that the existence of small wounds enormously increased the chance of infection. The presence of moisture was essential for infection; if the leaves were allowed to dry during the experiment the growth of the fungus was checked, a ring of callus was formed and the diseased area became occluded.

CONTROL

Miss Wakefield stated that in the outbreak of this disease which had previously come to her notice almost complete control was obtained by ceasing to clip the hedge regularly and cutting it back only in the autumn, so that there was not a succession of young leaves to become infected. The Dumfriesshire hedge has now practically recovered from the attack: a portion of it was cut back severely in June 1932, and this part is now quite free from the disease, but the remainder, which has been clipped as usual once a year in June, still exhibits slight traces of infection.

Laboratory experiments showed that spraying with colloidal sulphur greatly reduced infection, although complete control was not effected.

SUMMARY

A leaf spot disease of cherry laurel is described. The infected areas eventually become occluded and fall out, leaving irregular holes in the foliage. The disease is caused by *Trochila Laurocerasi* (Desm.) Fr., the imperfect stage of which is proved to be *Gloeosporium phacidiellum* Grove. Infection takes place on the young leaves, most commonly through wounds, and a moist atmosphere is essential for the development and spread of the disease. Control can be effected by spraying with colloidal sulphur during spring and early summer, and by cutting back the bushes only in the autumn, not at short intervals during the summer as is frequently the practice.

The writer wishes to acknowledge her indebtedness to Dr G. H. Pethybridge for valuable suggestions during the course of this work, also to Dr C. E. Foister for the photographs reproduced on Pl. XXXI.

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EXPLANATION OF PLATE XXXI

Both figures refer to *Trochila Laurocerasi* on cherry laurel.

Fig. 1. Natural infection. Infected areas, bearing acervuli and apothecia, becoming separated from the leaves.

Fig. 2. Artificial infection. Earlier stage, showing discoloured areas (marked X) sharply delimited by a thickened ring of callus and a second ring developing outside the first.

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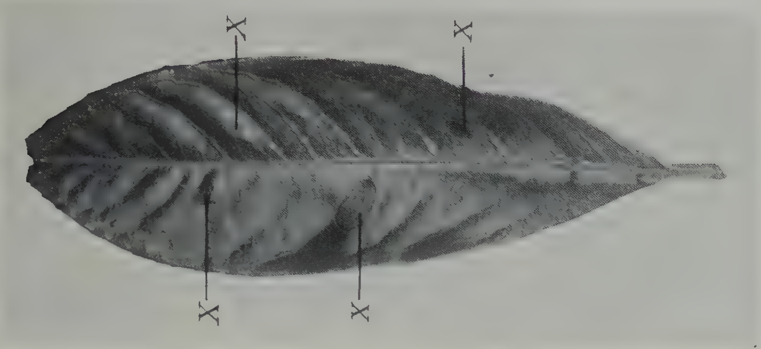

Fig. 2

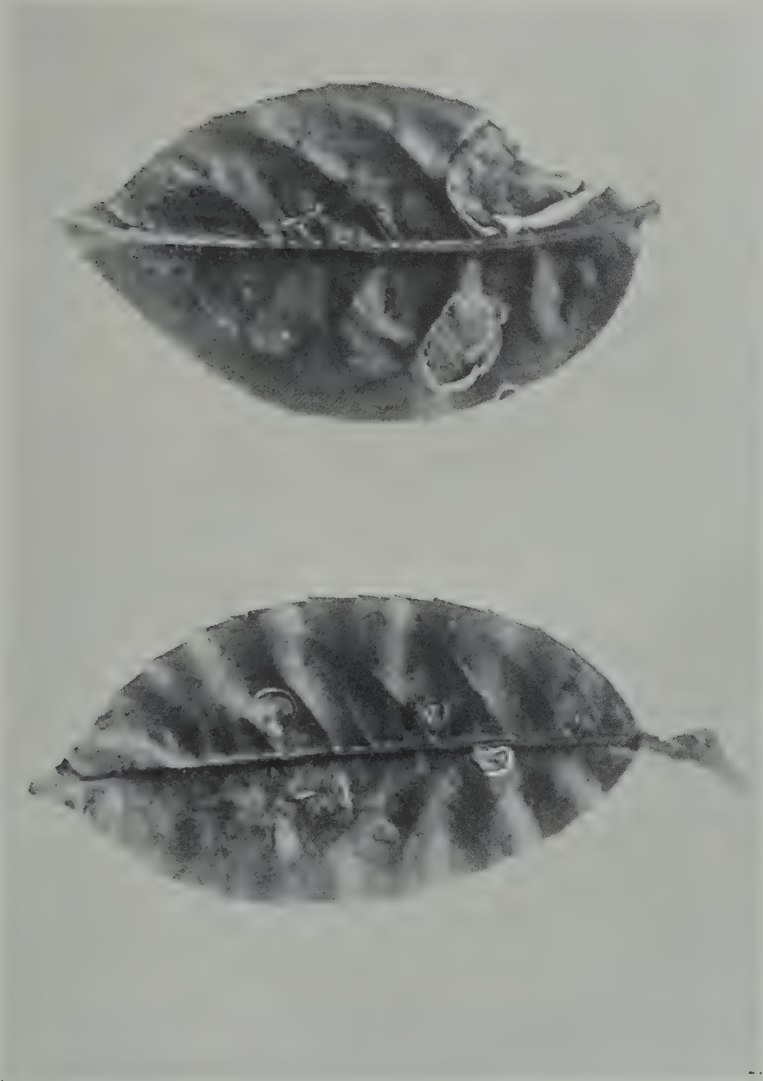

Fig. 1

GREGOR.—A Disease of Cherry Laurel caused by *Trochila Laurocerasi* (Desm.) Fr.
(pp. 700–704)

OBSERVATIONS ON THE BLACK-STEM DISEASE OF LUCERNE IN BRITAIN

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(With Plate XXXII and 4 Text-figures)

Introduction

This disease of lucerne, first reported from the trial grounds of The National Institute of Agricultural Botany, Cambridge, during April 1934, was brought to the notice of the senior author by Dr Dillon-Weston. As the disease seemed to be of some importance and to have escaped attention hitherto in this country it was decided to investigate it. It soon became apparent that a species of *Ascochyta* (or *Phoma*) was probably the cause of the disease, which was closely similar in all respects to the black-stem disease of alfalfa (lucerne) described in the United States by Johnson & Valleau (3), who attributed it to *P. Medicaginis* Malbr. & Roum. Consequently, cultures of species of *Ascochyta* and *Phoma* pathogenic to lucerne were sought from the United States for comparison with the British fungus. The following cultures were kindly sent: (?) *Phoma Medicaginis* Malbr. & Roum. (Nos. 900 and 1111) by Dr F. R. Jones; *P. Medicaginis* Malbr. & Roum. by Dr E. M. Johnson; (?) *Ascochyta imperfecta* Peck (No. 1135) by Dr F. R. Jones; *A. imperfecta* Peck (isolated from lucerne at Kelowna, British Columbia) by Dr R. Sprague. Exsiccata were also obtained of *A. Medicaginis* Fuck. (det. Fuckel) from Mr J. Ramsbottom of the British Museum (Natural History), of *A. imperfecta* Peck (collected in Ohio, 1927 and det. Sprague), both from Dr Shear of Washington and from Dr Sprague, and of the same fungus (collected by F. C. Stewart in the United States, June 1908) from Dr F. R. Jones. The latter was part of the type collection in the New York State Museum. In addition, Dr Jones sent material of (?) *Phoma Medicaginis* Malbr. & Roum. collected by himself at Monroe, Wisconsin, May 1931.

The disease was studied in the trial plots of lucerne at the National Institute of Agricultural Botany and in the leys on the University Farm. The disease was also found in Hertfordshire, Suffolk, Norfolk and Bedfordshire. It is probably widespread in this country, and Miss

K. Sampson stated in a letter to Dr Dillon-Weston, April 1934, that she had noticed a similar disease of lucerne in Wales.

FIELD OBSERVATIONS

The most badly affected lucerne plot at the National Institute of Agricultural Botany, which was examined in detail on 11 May 1934, had been grown from seed obtained from Iraq. The "stand" was very poor; some plants were dead or dying and many shoots were wilted. The following symptoms were observed:

On the stems. Sunken lesions of irregular shape, often at the base of the stipules, ¼–2½ in. long, varying in colour from dark brown to black, with a light brown centre (Pl. XXXII, fig. 1). Pycnidia were usually present in the old lesions. The lesions partly or wholly girdled the stems, sometimes forming "cankers" thereon, and frequently caused wilting and death of the shoots.

On the petioles. Elongated blackish lesions similar to those on the stems; necrotic zones at the base caused the leaves to become prematurely yellow and to wither.

On the leaf laminae. Lesions of variable size, round or irregular in shape, either on the edge or in the middle of the leaflets, dark brown or black in colour, occasionally zoned, sometimes becoming paler in the centre with age and sometimes surrounded by a yellowish halo (Pl. XXXII, figs. 2 and 3). The pycnidia were obscure and were not always present in the leaf lesions. Leaves bearing many spots rapidly turned yellow and withered.

These symptoms agree precisely with those of the black-stem disease of alfalfa (lucerne) in Kentucky described by Johnson & Valleau (3). We would like to emphasize the serious nature of the stem lesions, including the "cankers", which may lead to death of the shoots.

There is evidence in this country, similar to that given by Johnson & Valleau in the United States, that a moist spring greatly favours the spread of the disease. About the middle of April 1935, there was heavy rain at Cambridge, and by the end of the month black-stem injury had spread throughout the lucerne stands on the University Farm. The intensity of attack depends partly on the nature of the lucerne ley, the injury being greater in a pure crop than in lucerne which is a component of a "seeds" mixture.

Three types of leaf infection can be distinguished in the pure ley. In the first, the spots are few in number and large in size. The second is characterized by a unilateral attack of the leaflets, which may be due to them being folded when infection occurs. The third type of infection consists of uniformly small spots.

It has been the practice on the University Farm to cut the first crop of the season early, i.e. at the end of May, which has the advantage of delaying flowering. According to investigators abroad, the disease should not reappear after the first crop of the season has been cut. On the University Farm, however, where the crop was grown as a pure ley, the disease reappeared in 1935 about a month after the first crop had been cut, but less severely than before, especially as regards stem injury. Wet weather in early June doubtless favoured the development of the disease in the second crop. In contrast to the practice on the University Farm may be cited the treatment on a farm at Bridgham, Norfolk, where the first cutting was delayed until 25 June 1935, with the object of obtaining a particularly heavy yield of herbage. In early June it was noticed that the "plant" had failed to "come on"; subsequent examination showed that many shoots had been killed by black-stem injury, and there was clear indication of the damage that could be caused by the disease when unchecked by early cutting of the crop.

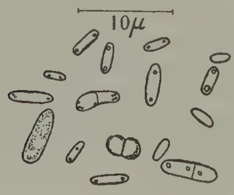
Relative susceptibility of lucerne strains

No strain of lucerne grown at Cambridge was free from the disease during the summers of 1934–5, but the one from Iraq was undoubtedly the most susceptible. At Bridgham, Norfolk, where there was a four-year-old trial of lucerne strains conducted by the National Institute of Agricultural Botany, the strains were placed in the following order of decreasing susceptibility in 1935: (1) Medanos, (2) English grown and Grimm, (3) Provence, Marlborough and Hungarian.

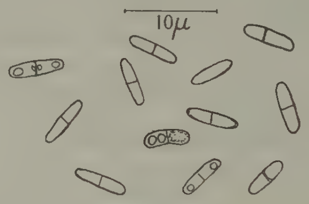
DESCRIPTION OF THE FUNGUS WHICH CAUSES BLACK-STEM DISEASE OF LUCERNE

In the stem and leaf lesions globose, ostiolate pycnidia, averaging 140μ in diameter and varying from light to dark brown in colour, are produced. The pycnospores are hyaline, oval or cylindrical with rounded ends, straight or slightly curved, continuous or uniseptate. The percentage of uniseptate spores in a pycnidium is extremely variable, and in some collections in the field and in certain cultures all the spores were unicellular. Old spores often contain two or more small guttules and they may be slightly constricted at the septum. Spores from a leaf of naturally infected Iraq lucerne are shown in Text-fig. 1, and spores from a stem of the same variety are shown in Text-fig. 2. Many unicellular spores undergo septation prior to germination in water, but this is less marked in 2 per cent dextrose and does not occur in carrot decoction.

The spore size of this fungus is also extremely variable. The degree of variation in spore size has been gauged by selecting uniform samples of spores which have developed under different conditions. In each collection one hundred spores from a single pycnidium were measured by means of an oil-immersion lens and a Leitz Filar micrometer eyepiece. A selection of the measurements of the spores from lucerne and culture media is given in Table I, together with measurements of the spores of *Ascochyta Medicaginis* Fuck. and *A. imperfecta* Peck from herbarium material. The spores of the fungus on malt-extract agar are somewhat larger than on Dox's agar.



Text-fig. 1.



Text-fig. 2.

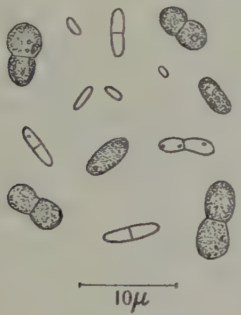
Table I

Measurements, etc., of spores from hosts and culture media

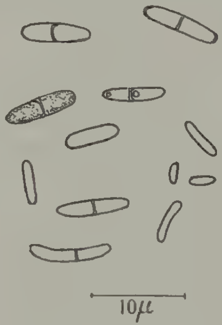
Source of spores	Percentage septate	Length			Width		
		Mean	Standard deviation	Range	Mean	Standard deviation	Range
Natural infection:							
1. *A. Medicaginis* Fuck., det. L. Fuckel, 1886, leaf	Nil	5·5	0·57	4·0– 7·0	2·2	0·16	1·8–2·9
2. *A. imperfecta* Peck, coll. and det. Sprague, 25. iv. 27, leaf (U.S.A.)	35	11·4	2·18	7·5–15·0	3·5	0·47	2·6–5·1
3. Hungarian lucerne, upper part of stem, 13. vii. 35	Nil	6·4	1·22	4·0–10·0	2·6	0·24	2·1–3·5
4. Hungarian lucerne, lower part of stem, 13. vii. 35	68	7·1	0·72	5·0– 9·4	2·7	0·23	2·1–3·2
5. Hungarian lucerne, leaf, 15. vii. 35	Nil	7·3	0·79	5·0– 9·0	2·8	0·29	2·3–3·7
Artificial infection:							
6. Iraq lucerne, leaf, 28. ix. 34	Nil	6·7	1·20	3·7– 9·1	2·5	0·31	1·7–3·1
7. Provence lucerne, stem, 24. vi. 35	34	10·8	1·83	6·2–15·0	3·3	0·37	2·0–4·2
8. Provence lucerne, stem, 24. vi. 35	Nil	5·7	0·71	4·0– 7·5	2·4	0·24	1·7–3·2
Cultures:							
9. Dox's agar, 6 months at 5° C.	7	9·5	2·75	5·0–17·8	3·0	0·44	1·8–4·0
10. Dox's agar, 1 month at room temperature	Nil	5·0	0·74	3·5– 7·0	2·2	0·28	1·5–3·1

The fungus isolated from the black-stem disease of lucerne at Cambridge grows readily and produces fertile pycnidia on most media. Many isolations have been made from stems and leaves, both with unicellular and with uniseptate spores, and they are all essentially the same in cultural behaviour. There is often a great preponderance of unicellular spores in the cultures. The following description refers to cultures, 3 weeks old, in Petri dishes exposed to light at room temperature, except where otherwise stated:

On potato-dextrose agar the superficial mycelium is greyish olive or olive in colour as seen with the naked eye, and the embedded mycelium (as seen on the reverse side of the plate) is black or olive-black with an irregular whitish margin. There is a somewhat pronounced staling effect. The abundant pycnidia, which at first are covered with an evanescent mycelial felt, are embedded in a carbonaceous matrix consisting of dark brown hyphae and chlamydospore-like bodies. The spore exudate is pale pink and consists chiefly of unicellular spores often containing two or more small guttules.



Text-fig. 3.



Text-fig. 4.

On 5 per cent malt-extract agar the superficial mycelium is markedly olive-green, and the embedded mycelium is black as seen with the naked eye. Numerous pycnidia are formed in the mycelial matrix, which consists chiefly of olive-brown hyphae.

On Dox's agar the superficial mycelium is greyish and the embedded mycelium is black with an irregular whitish margin. There is a somewhat pronounced staling effect. Numerous pycnidia are formed in the mycelial matrix which consists chiefly of dark-coloured hyphae.

On nutrient gelatin growth is rapid and lobate, the medium being liquefied. Pycnidia are not formed on this medium. *Ascochyta Pisi* Lib. also liquefies gelatin.

On sterilized lucerne and *Melilotus* stems in test-tubes, inoculated at the top, a loose cottony mycelium develops which grows downwards for about half an inch; the lower part of the stem later becomes covered with blackish pycnidia.

The spores become increasingly uniseptate in old cultures. Spores from a culture kept at room temperature for 5 months are shown in Text-fig. 3, and spores from a culture of the same isolation kept at 5° C. for 6 months in Text-fig. 4.

Cultures of *Ascochyta Pisi* Lib. were grown side by side with cultures of the fungus isolated from the black-stem disease of lucerne. *A. Pisi* is an entirely different fungus.

It will be convenient now to consider the cultural characteristics of the fungi from lucerne sent from the United States for comparison with the British fungus, viz. (?) *Phoma Medicaginis* Malbr. & Roum. (Nos. 900 and 1111) from Dr F. R. Jones, *P. Medicaginis* Malbr. & Roum. (No. 230:18) sent by Dr E. M. Johnson as the cause of black-stem disease of lucerne in Kentucky, (?) *Ascochyta imperfecta* Peck (No. 1135) from Dr F. R. Jones, and *A. imperfecta* Peck from Dr R. Sprague. All of these, with the possible exception of No. 900 from Dr Jones, look essentially the same as the British fungus when grown on potato-dextrose, malt-extract and Dox's agars and on nutrient gelatin in Petri dishes, and the spores vary in size and septation in the same way. No. 900 is somewhat lighter in colour on Dox's agar as regards the submerged mycelium and does not stale on this medium as do the others; on malt-extract and potato-dextrose agars and on nutrient gelatin it is identical with the rest. It is probably only a slightly different strain in comparison with the other cultures. White, dendroid crystal aggregates are formed in the medium in old cultures of all these fungi on Dox's agar.[1] We have come to the conclusion that all these American cultures belong to the same species of fungus as that which causes black-stem disease of lucerne in this country. Dr F. R. Jones, to whom a culture of the British fungus was sent, kindly informs us that he is now of the opinion that all these fungi are identical.

IDENTIFICATION OF THE FUNGUS

At an early stage in this investigation we decided that it would be preferable to refer the fungus which causes the black-stem disease of lucerne in this country to the genus *Ascochyta*, rather than to *Phoma*, because of the fairly frequent occurrence of some uniseptate spores. It seemed probable that the fungus was *Ascochyta imperfecta*, first described by Peck (5) in the United States, and investigated by Sprague (7) in 1929.

---

[1] These crystals have been kindly examined by Dr J. D. H. Wiseman, Department of Mineralogy, Cambridge University, who reports that they are probably a sodium phosphate the constants of which have not hitherto been determined.

On the other hand, Johnson & Valleau (3) were inclined to refer to *Phoma Medicaginis* Malbr. & Roum. the fungus causing black-stem disease of lucerne in Kentucky, which is undoubtedly identical in symptoms with the British disease. In their paper they state that a few spores of their fungus were uniseptate. They make no reference to *Ascochyta imperfecta* Peck.

As stated previously, cultures of Johnson & Valleau's fungus, of other collections of *Phoma Medicaginis* and of *Ascochyta imperfecta* from North America are indistinguishable from the British fungus, and we are disposed to refer them all to *Ascochyta imperfecta* Peck. On this basis, *Phoma Medicaginis* Malbr. & Roum. (if the American determination is correct) is a synonym of *Ascochyta imperfecta* Peck. The spore characters of the dried material of *A. imperfecta* Peck collected by Dr Sprague in Ohio in 1927 and determined by him, and of the type material of this fungus from the New York State Museum (collected by F. C. Stewart in 1908), which we have examined, agree well with those of some of our British collections (e.g. Table I, 2 and 7). Material of a fungus on lucerne, collected by Dr F. R. Jones at Monroe, Wisconsin, in 1931 and then provisionally named by him as *Phoma Medicaginis* Malbr. & Roum., is also essentially the same as the British fungus. It was from this material that Dr Jones established culture No. 900 (referred to above).

With regard to *P. Medicaginis* Malbr. & Roum., C. Roumeguère and A. Malbranche described it as a new species on dry stems of *Medicago sativa* collected at Rouen in 1885. It was distributed as No. 3675 of C. Roumeguère's *Fungi Gallici Exsiccati*. A. Malbranche (*Rev. Mycol.* 1886, VIII, 91) refers to it as follows: "3675. *Phoma medicaginis* Malbr. & Roum. sp.nov. (*Ph. herb.* f. *medicaginis* in Herb.) diffère du type par des sporules oblongues, hyalines, plus petites, $3–7 \times 1–2{\cdot}5\,\mu$." In this description *Ph. herb.* f. *medicaginis* doubtless refers to *Phoma herbarum* Westend. f. *medicaginis* Fuckel, which was issued by Fuckel as No. 580 in *Fungi Rhenani* and was described in his *Symbolae Mycologicae*, 1869, p. 134. The specimen of *Ph. herbarum* Westend. f. *medicaginis* (*Fungi Rhenani*, No. 580) in the British Museum (Natural History) has been examined, and although few spores were found it appears to be the same as the fungus under consideration in this paper. An effort has been made to obtain the type specimen of *Phoma Medicaginis* Malbr. & Roum, but Dr Heim of the Paris Museum informs us that no specimens of this fungus can now be found in the Paris herbaria. In view of the fact that we have been unable to examine the type specimen we cannot say with certainty that *P. Medicaginis* Malbr. & Roum. is identical with *Ascochyta imperfecta* Peck. It has been established, however, that the American fungus on lucerne which has received the name of *Phoma Medicaginis* Malbr. & Roum. is identical with *Ascochyta imperfecta* Peck.

Phoma vulgaris Sacc. (Saccardo, *Sylloge Fungorum*, III, 119) and *P. anceps* Sacc. (*loc. cit.* p. 120) are also recorded on lucerne stems, but their descriptions are meagre and no useful comparison can be made between them and the fungus under consideration here.

Diplodina Medicaginis Oud. (Saccardo, *Sylloge Fungorum*, XVIII, 351) reported on *Medicago* sp. in Holland may be identical with *Ascochyta imperfecta* Peck, but we have not yet been able to examine it.[1]

Table II

Summary of spore measurements of fungi on lucerne, etc.

	Species	Mean (μ)	Range (μ)
1.	**Ascochyta Medicaginis* Fuck.	5·5 × 2·2	4·0– 7·0 × 1·8–2·9
1 *a*.	*A. Medicaginis* Fuck.	6·0–7·0 × 2·5	5·0– 9·0 × 2·0–3·0
2.	*Phyllosticta Medicaginis* (Fuck.) Sacc.	—	5·0– 7·0 × 2·5–3·0
2 *a*.	*P. Medicaginis* (Fuck.) Sacc.	—	8·0–14·0 × 3·0–4·5
2 *b*.	” ”	5·0–6·0 × 1·0	—
3.	*Ascochyta Medicaginis* Fuck.	11·8 × 3·8	8·0–14·7 × 2·3–4·7
4.	*A. imperfecta* Peck	—	6·0–15·0 × 2·5–4·0
4 *a*.	”	10·6–15·4 × 2·3–4·3	—
4 *b*.	”	12·1 × 3·4	—
4 *c*.	”	11·4 × 3·5	7·5–15·0 × 2·6–5·1
4 *d*.	”	5·7–10·8 × 2·4–3·3	3·7–15·0 × 1·7–4·2
5.	*A. Pisi* Lib. var. *Medicaginis* Sacc.	14·0 × 5·5–6·6	—
6.	*Phoma vulgaris* Sacc.	8·0–10·0 × 2·5–3·0	—
7.	*P. herbarum* West. f. *Medicaginis* Fuck.	10·0 × 3·0	—
8.	*P. Medicaginis* Malbr. & Roum.	—	3·0– 7·0 × 1·0–2·5
9.	” ”	—	5·0–12·0 × 1·5–2·5
10.	†*Ascochyta Medicaginis* Bres.	—	16·0–26·0 × 3·5–5·0

* The host was *Medicago falcata*. † The host was *Medicago lupulina*

Explanation of Table II

1. Authors' measurements from British Museum exsiccata, det. L. Fuckel.
1 *a*. Determined by Jones(4).
2. Determined by Corneli(1).
2 *a*. Determined by Corneli(1) after spores had swollen in water.
2 *b*. Determined by Grove(2).
3. Determined by Rosella(6).
4. Determined by Peck(5).
4 *a*. Determined by Sprague(7) as the range of means of samples.
4 *b*. Determined by Sprague(7) as the grand average of all the means in 4 *a*.
4 *c*. Authors' measurements from exsiccata from Ohio, det. Sprague.
4 *d*. Authors' aggregate of all the spore measurements of the fungus causing black-stem disease of lucerne in Britain.
9. Determined by Johnson & Valleau(3).

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[1] Through the kindness of Prof. B. H. Danser of Groningen the type specimen has now been examined. *Diplodina Medicaginis* Oud. is undoubtedly identical with *Ascochyta imperfecta* Peck.

In 1929 Rosella (6) described a disease of lucerne in France which he attributed to *A. Medicaginis* Fuck., and in 1932 Corneli (1) investigated a similar disease in Italy which he referred to *Phyllosticta Medicaginis* (Fuck.) Sacc. Unfortunately, it has been impossible to obtain material of these diseases or cultures of the fungi with which these authorities worked. The symptoms of the diseases they describe are similar to those of the black-stem disease in Britain, and it may well be that the causative fungus is the same, viz. *Ascochyta imperfecta*. For instance, Corneli states that the spores of his fungus are occasionally uniseptate and that unicellular spores mostly become septate when germinating in water, features which are characteristic of the British fungus. *A. Medicaginis* Fuck. is a synonym of *Phyllosticta Medicaginis* (Fuck.) Sacc., which Jones (4) has shown to be the conidial stage of *Pyrenopeziza Medicaginis* Fuck. Jones (4) points out that a characteristic feature of the fungus is the regular linear arrangement of the pycnidia in the affected leaves, which we can confirm after examining material of *Ascochyta Medicaginis* Fuck. in the British Museum. Neither Corneli nor Rosella refers to this feature.

Ascochyta Pisi Lib. var. *Medicaginis* Sacc. should probably be included in *A. imperfecta* Peck, as suggested by Sprague (7).[1]

A. Medicaginis Bres. is specific to *Medicago lupulina* and is probably different from *Ascochyta imperfecta* Peck.

A summary of the spore measurements of most of the fungi mentioned above is given in Table II. In view of the wide range of the spore measurements of British and American field collections and cultures of *Ascochyta imperfecta* no great reliance can be placed merely on such measurements in the determination of these species.

INOCULATION EXPERIMENTS

In order to test the pathogenicity of the fungus isolated from the lesions, lucerne plants which had been grown from seed in a greenhouse were inoculated with a spore suspension from a culture, and were then kept under a bell-jar in a cool place. All inoculations, both of wounded and unwounded plants, were successful, lesions appearing on the stems and leaves in 6 days. Wounding facilitated the progress of the fungus during the early stages of infection. The fungus was reisolated from the lesions. The stems of older plants were also inoculated, after surface sterilization, by applying wads of cotton-wool saturated with a spore suspension of the fungus and covered with tinfoil. A month later about half of the inoculated plants were found to be heavily infected; some stems were cankered and others were killed nearly to the base. The fungus was again reisolated from the pycnidia.

---

[1] Dr F. R. Jones has suggested that *Rhabdospora* (*Septoria*) *allantoidea* (B. & C.) Sacc. (*Sylloge Fungorum*, III, 586), collected by Michener in Pennsylvania in 1874, may be identical with *Ascochyta imperfecta* Peck. The type specimen in the Kew herbarium has been examined. It may be the same fungus, but we cannot be certain as we could not obtain a good preparation of the spores.

Infection of lucerne plants about 3 months old was also achieved in the open. In this experiment a row of each of the following strains of lucerne was sprayed with a spore suspension on 11 August 1934, another row of each being kept as a control: Iraq, Provence, English grown, and Hungarian. Six weeks later all strains showed typical brownish black lesions. The Iraq strain was more severely affected than the others and some of the stems had been killed. The fungus was reisolated from the pycnidia. At this date the control rows were healthy, but they subsequently became infected, presumably from the inoculated rows.

In order to test the host range of the fungus the following leguminous plants were inoculated simultaneously: *Pisum sativum* var. English Wonder, *Vicia Faba*, *V. sativa*, *Trifolium pratense*, *T. repens*, *Medicago sativa*, and *M. lupulina*. For comparison a parallel series of inoculations was made with *Ascochyta Pisi* Lib., freshly isolated from peas. The plants were sprayed with spore suspensions and were kept under bell-jars The results were as follows:

Pisum sativum. Infection by *Ascochyta Pisi*, but not by *A. imperfecta*.

Vicia Faba. No infection.

Vicia sativa. No infection by *Ascochyta Pisi*, but slight spotting of the leaves was caused by *A. imperfecta*.

Trifolium pratense. No infection by *Ascochyta Pisi*, but lesions similar to those on lucerne were produced on the laminae and petioles by *A. imperfecta*, together with some leaf scorch.

Trifolium repens. No infection.

Medicago sativa. No infection by *Ascochyta Pisi*, but typical lesions produced by *A. imperfecta*.

Medicago lupulina. No infection by *Ascochyta Pisi*, but lesions similar to those on lucerne were produced by *A. imperfecta*.

Thus *Ascochyta imperfecta* can infect *Trifolium pratense* under artificial conditions. This is in agreement with the results of Sprague(7). It is of interest also that Johnson & Valleau(3) were able to infect red clover with the fungus which causes black-stem disease of lucerne in Kentucky.

Finally, a series of inoculations of young Iraq lucerne plants in a greenhouse was carried out simultaneously with a culture of the British fungus and with the five cultures sent from North America. All gave rise to identical types of lesions, which produced pycnidia and spores indistinguishable from one another. This is further proof that the five American cultures belong to the same species as the British fungus.

DISCUSSION

Black-stem disease of lucerne has been under observation by the authors for two years. It is somewhat remarkable that the disease has not hitherto been recorded in this country. This may be due to confusion with the disease caused by *Pseudopeziza Medicaginis* (Lib.) Sacc., although the symptoms of the two diseases are distinct. In a wet spring, such as that of 1935, the loss of herbage caused by black-stem disease must be considerable. The disease certainly assumes economic importance when lucerne is grown on a large scale for manufacturing lucerne-meal. Such a venture has recently been started at Southacre, Norfolk, where more than 2000 acres are being grown. One of us was asked to investigate the cause of the discoloration and death of the shoots there during the summer of 1935. The damage proved to be entirely due to *Ascochyta imperfecta*. As the quality of the lucerne-meal depends on the colour of the foliage and the leaf/stem ratio, the serious nature of this attack can be appreciated.

The fungus undoubtedly overwinters in dead lucerne shoots, whence infection of the new shoots occurs in spring. It has been repeatedly isolated from dead lucerne stems during winter and early spring. Any measure which removes these dead shoots is likely to reduce primary infection in the new growing season. Johnson and Valleau (3) state that winter grazing by sheep achieves this in Kentucky, and similar benefit would probably accrue in this country.

Observations here indicate, however, that a simple method of control of black-stem disease lies in cutting the first crop of herbage early in the spring. In this way the first crop is harvested before leaf and stem injury is severe. Later crops in the same season are never so seriously affected as is the first crop when the cutting of this is delayed. It is the practice on the Cambridge University Farm to cut the first crop of the season at the end of May.

SUMMARY

1. Black-stem disease of lucerne, which may result in a serious loss of herbage, is recorded in this country for the first time.

2. The disease first appears on the young shoots early in spring. It is characterized by large, irregular, brownish or blackish lesions on the leaves, petioles and stems. Severe attack of the stems may involve the formation of "cankers" and may lead to wilting and death of the shoots.

3. The cause of the disease is a species of *Ascochyta* which has been determined as *A. imperfecta* Peck. The dimensions of the spores and the percentage of uniseptate spores in the pycnidia are extremely variable. Some field collections and cultures of the fungus possess only "phomoid" (i.e. unicellular) spores.

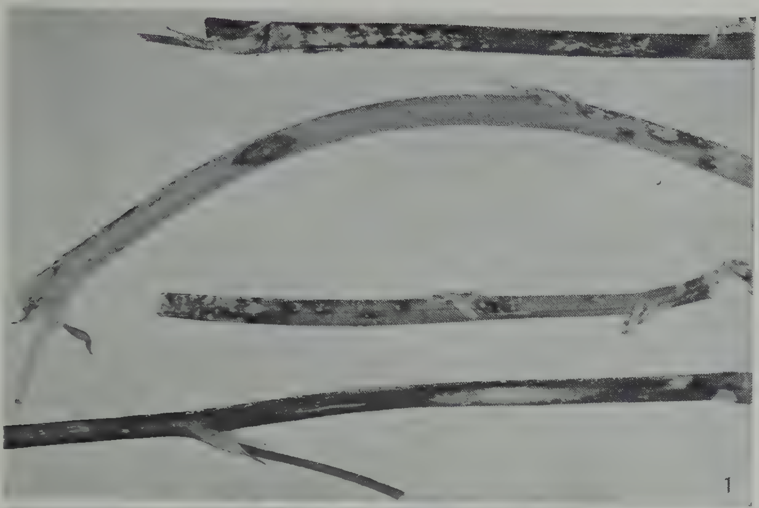
4. The characteristics of the fungus in culture are described. It has been compared with cultures of *Ascochyta imperfecta* Peck and *Phoma Medicaginis* Malbr. & Roum. from North America. All the American cultures agree with the fungus isolated from black-stem disease of lucerne in Britain. It is concluded that the fungus which has been referred to as *P. Medicaginis* Malbr. & Roum. in the United States is synonymous with *Ascochyta imperfecta* Peck. This opinion is substantiated by the formation of identical types of lesions on lucerne by the cultures sent from North America and by the British fungus in simultaneous inoculation experiments.

5. Herbarium material of *Phoma Medicaginis* and *Ascochyta imperfecta*, including type material of the latter, sent from the United States has been compared with British material of the black-stem disease of lucerne. It is concluded that the fungus in all these collections is the same, viz. *A. imperfecta* Peck.

6. Under artificial conditions *A. imperfecta* can also infect *Medicago lupulina* and *Trifolium pratense*, but it has not been found on these hosts in the field.

7. *Ascochyta imperfecta* overwinters by means of pycnidia in dead lucerne stems.

8. The black-stem disease of lucerne can be held in check by early cutting of the first crop each season.



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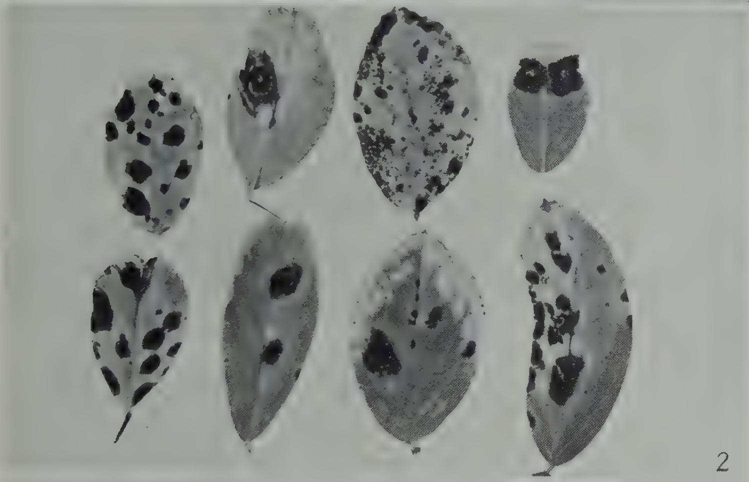

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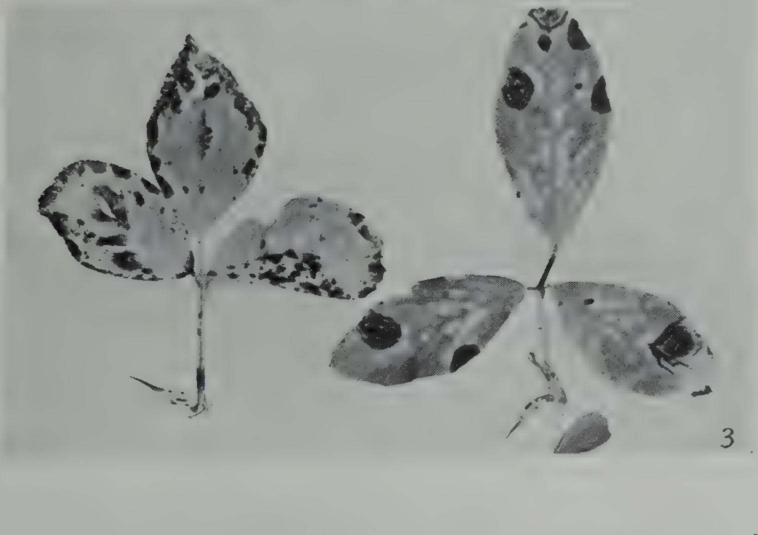

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TOOVEY, WATERSTON AND BROOKS.—Observations on the Black-stem Disease of Lucerne in Britain (pp. 705–717)

Acknowledgements

We are greatly indebted to Dr C. L. Shear, Dr F. R. Jones, Dr R. Sprague and Dr E. M. Johnson for kindly sending us specimens and cultures from the United States. We are especially grateful to Dr F. R. Jones who has simultaneously compared many of the collections and cultures referred to in this paper and who has been of immense assistance to the senior author in corresponding freely on the comparison which has been made between this disease of lucerne in Britain and the United States. It is largely due to his generous help that the identity of the disease in the two countries has been established. We should also like to thank Mr J. Ramsbottom of the British Museum for allowing us to see certain herbarium specimens and for help in matters of nomenclature.

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EXPLANATION OF PLATE XXXII

Fig. 1. Black-stem disease of stems of Iraq lucerne, April 1935. × 1.

Fig. 2. Leaflets of Iraq lucerne with various types of lesions caused by *Ascochyta imperfecta* Peck. × 1.

Fig. 3. Leaves of Iraq lucerne showing the two extreme types of infection met with in the field. × 1.

(*Received* 23 *March* 1936)

THE CONTROL OF COMMON SCAB (*ACTINOMYCES SCABIES* (THAXT.) GÜSS.) OF THE POTATO BY TUBER DISINFECTION

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I. INTRODUCTION

General

THE injury caused by common scab, *Actinomyces scabies* (Thaxt.) Güss., to the potato tuber may be regarded from different aspects. From the culinary point of view the actual loss is not great except in the case of badly affected samples which necessitate a deeper peeling of the tubers. Nevertheless, the fact remains that scabbed potatoes are unsightly and unpopular with the housewife. Many farmers dispose of the most severely affected portion of the crop by feeding it to livestock, a practice which is economically unsound except in years of low prices. During storage there is a greater liability of scabbed tubers becoming affected with rot-producing organisms such as *Fusarium* spp., while, irrespective of this, it has been shown (12) that scabbed tubers lose more weight during storage than sound tubers.

The disease, however, is most serious in the case of tubers used for seed. The possibility of introducing the disease by the use of scabbed seed must be avoided wherever practicable, and the experiments described in this paper were undertaken with the object of determining to what extent common scab may be controlled in the resultant crop by seed tuber disinfection. A survey of the literature dealing with the work carried out in connexion with the control of this disease indicates that, apart from seed tuber disinfection, methods involving soil treatment and the use of resistant varieties have been investigated and before proceeding to a description of the tuber disinfection experiments a short summary is given dealing with control methods in general.

Green manuring

The strains of *Actinomyces scabies* which cause common scab are widespread and have been recorded in many types of soil and under a wide range of environmental conditions. It would appear, however, that the disease is most prevalent on light gravelly soils of low humus content with an alkaline or slightly acid reaction. Temperature and moisture also play important roles; a high temperature and a low moisture content of the soil being predisposing factors in the production of a severely scabbed potato crop. Millard (18), from the study of the hydrogen-ion concentration of a large number of soils in which scab was known to be prevalent, came to the conclusion that the amount of humus present is of more importance than the soil reaction. He also showed that a very appreciable amount of control could be obtained by green manuring, which entails the ploughing in of a green crop (rye, mustard, vetches, etc.) or the application of green material (grass cuttings, spent hops, etc.) to the furrows before planting. Millard explained the beneficial effect of green manuring by his "Preferential food" hypothesis. Later Sanford (21) suggested that the success of green manuring is due to the inhibiting influence of certain micro-organisms on the development of *A. scabies*. Pot experiments by Millard & Taylor (19) indicated that the failure in some cases of green manuring to control scab is due to the absence from the soil of certain saprophytic species of *Actinomyces*, the growth of which is encouraged by green manuring and leads to the suppression of the scab organisms.

Apart from the uncertainty of control the adoption of green manuring in ordinary farm practice adds to the cost of production and, so far, the use of this method has not become widespread.

Use of sulphur

The control of scab by the application of sulphur to the soil has been investigated in the United States and Canada. The results, however, have been even more inconsistent than those from green manuring, and sulphur has not proved effective on all soils and under all conditions. The beneficial effects of sulphur would appear to be mainly due to the increased acidity of the soil following its use, the sulphur being oxidized by micro-organisms present in the soil. Martin (14) points out that when these organisms are absent it is necessary to supply them and he has obtained better control by the use of sulphur inoculated with the appropriate organisms than with similar amounts of uninoculated sulphur. He is also of the opinion (15) that, apart from the increase in acidity, the disinfectant action of sulphur also plays a part in the reduction of scab. The failure of sulphur has been connected with the time of application, and Taylor (26) has obtained control when the sulphur was applied after the spring ploughing but not before ploughing. Barrus & Chupp (2) report that sulphur is more effective on light soils. Dippenaar (32) obtained the best control by an application of sulphur combined with liberal irrigation of the soil.

Where green manuring alone and sulphur alone have failed a combination of the two treatments has proved effective (29), but failures by this method have also been reported (30). In addition to the somewhat erratic results obtained by the use of sulphur there is a serious danger of reduction in yield following its application. This reduction has been prevented (31) by the combined treatment of sulphur and green manuring, but the costliness of the method is a drawback.

Use of lime and fertilizers

The effect of lime and fertilizers is by no means a straightforward problem. In general the use of physiologically acid fertilizers, e.g. ammonium sulphate and superphosphates, tends to reduce the incidence of the attack, while substances such as lime and basic slag cause an increase. According to Millard (18) the action of lime on scab production may depend upon the soil reaction, its effect in neutral soils being negligible, while in the case of acid soils its application aggravates the occurrence of the disease unless the soil is rich in humus. Sibilia (25) in Italy and Schlumberger (24) in Germany both record experiments where a reduction of scab was effected by the application of lime. Bolley (5) and Rang (20) obtained some control with calcium cyanamide, but Eichinger (7) found an increase in scab when this substance was used.

Soil disinfection

The effect of sulphur and artificial fertilizers on the incidence of scab is of an indirect nature and is considered to be due to a resultant change in the hydrogen-ion concentration of the soil. A method more direct in its action is the incorporation in the soil, before planting, of small quantities of certain mercury compounds with the object, evidently, of disinfecting the soil. Martin (16) has obtained very promising results with mercurous chloride (calomel), mercuric oxide (yellow oxide of mercury) and certain organic compounds of mercury. Van der Slikke (27) obtained successful results by the treatment of the planting holes with 0·25 l. of a 0·1 per cent mercuric chloride solution. Flutchey & Muncie (8) as a result of three years' field and greenhouse trials report the failure of mercury compounds. Taylor (32) found that the use of inorganic and organic mercury compounds increased scab in the high limestone regions of New York State.

Summarizing, none of the numerous soil treatments adopted has been successful on all soils and under all conditions, the success or failure being, undoubtedly, closely connected with such factors as hydrogen-ion concentration, humus content of the soil, the presence of certain micro-organisms in the soil, moisture and soil temperature, any one or all of which may have a marked influence on the result.

Resistant varieties

The development of potato varieties highly resistant or immune to common scab would provide an ideal means for the prevention of the disease. This aspect of the problem has received attention in Germany and America. The results obtained indicate that some varieties are highly resistant, but these are not necessarily those which are most popular with the farmer. Until resistant varieties have been produced which are capable of replacing the popular susceptible varieties which are so much grown, other methods of control must warrant serious attention.

Seed tuber disinfection

The numerous reports on the efficacy of seed-tuber disinfection for the control of scab indicate that this method has not been more successful than those already considered. The disinfection of the seed tubers before planting ensures against the risk of introducing *A. scabies* into scab-free land by the planting of scabbed seed. The general opinion, however, is that owing to the ubiquity of *A. scabies* in the soil, infection through the medium of the soil is more probable than that arising from the planting of affected tubers, in which case seed treatment would not provide control.

Sanford (22) found that *A. scabies* grew vigorously in soil practically in contact with potato tubers which had received any of the standard potato seed treatments. His results suggest that the incidence of scab on a potato crop is wholly dependent on the natural infection of the soil. MacLeod & Hurst (13) maintain that seed treatment will give only partial control, and effective control necessitates a combination of seed treatment and cultural practices which will correct the soil conditions predisposing to the disease. Taylor (32) records an increase in scab in limestone regions when the seed tubers were treated with mercury compounds prior to planting. Under the same conditions he found that a formaldehyde treatment reduced the disease.

Since their introduction towards the end of the last century by Arthur (1) and Bolley (4) the mercuric chloride and formaldehyde steep methods of tuber disinfection have been employed extensively in the United States for the control of scab. Other methods of tuber disinfection have also been used and, although the results have often been disappointing, in many cases tuber disinfection has proved to be a very effective means of control. It was thought that this fact coupled with the extreme practicability of the method warranted an investigation under Northern Ireland conditions.

The work was commenced on a small scale in 1932 and, as very promising results were obtained, more extensive trials were carried out during the subsequent years. Formaldehyde and compounds of copper and of mercury were used as tuber disinfectants, and the first part of the investigation deals with the efficiency of these materials. During 1934 and 1935 a number of tests were laid down on farms in County Down and County Armagh where scab was known to be prevalent.

In all the experiments very severely scabbed seed tubers were planted so that the particular fungicide was subjected to a severe test. The usual routine farm methods of potato culture were followed. The potatoes were harvested at the usual time, towards the end of September or during October, and at harvest the tubers were divided into two classes, scabbed and non-scabbed, a tuber showing any scab infection being counted as scabbed. The percentage by weight of scabbed tubers was then calculated. In many cases a small amount of infection with powdery scab (*Spongospora subterranea*) was present on both the seed and the produce. The incidence of powdery scab was in all cases insignificant, and it was found to be unnecessary to differentiate between the two diseases.

II. THE HILLSBOROUGH EXPERIMENTS—COMPARISON OF FUNGICIDES

General

Much of the technique of tuber disinfection has been developed for the control of *Corticium Solani* (stem canker), but it is generally considered that a method which is effective in killing the sclerotia of this fungus on the potato tuber should be at least equally effective for the destruction of the common scab pathogene. Satisfactory control of scab has been obtained by both the dry and wet methods of tuber disinfection, but, on the whole, the dry treatments, which consist of dusting the tubers with such substances as sulphur, "formaldehyde dust" and various proprietary organic mercury dusts, have not been so successful as the wet treatments and for this reason were not used in these experiments.

The following experiments were carried out at the Agricultural Research Institute, Hillsborough, County Down. After treatment the seed was stored in sprouting boxes till planting time, when it was planted in land which had not borne a potato crop within living memory. The plots were arranged at random and in most cases were replicated. Each plot in most of the experiments consisted of one drill 12 yards long, the same number of "sets" being planted per plot.

Farmyard manure was not applied to any of the plots on account of the possibility of introducing scab-producing organisms through this medium. The manurial treatment given was 1 part sulphate of ammonia, 4 parts superphosphate and 1 part muriate of potash applied at the rate of 9 cwt. per acre.

The use of mercuric chloride and formaldehyde as tuber disinfectants

The formaldehyde and mercuric chloride steep methods as introduced by Arthur and Bolley consist of the immersion of the tubers for a period of 1·5–2 hours in a weak solution of the particular fungicide, formaldehyde being used at the concentration of 1 pint of formalin (40 per cent formaldehyde) to 30 gallons of water (i.e. 1 : 240) and mercuric chloride at a 0·1 per cent concentration. Where tuber disinfection has been successful for the control of scab these treatments have been regarded as efficient, but the time required for the actual treatment is a disadvantage. To reduce the time factor Melhus (17) devised the hot mercuric chloride and formaldehyde methods, where, by holding the temperature of the disinfecting solution at 48–50° C., the time of the steep was reduced to 5 min. The hot treatments, however, have the disadvantage of requiring more elaborate apparatus and also the necessity of maintaining an accurately controlled temperature. Cunningham (6) found that the addition of a small quantity of hydrochloric acid to the mercuric chloride solution greatly increased the effectiveness of the latter, and that a 5-min. steep in this acidulated solution was sufficient.

All the above methods are now recognized as standard for tuber disinfection, but a simpler method would find greater favour in farm practice.

Mercuric chloride and formaldehyde were used as 90-min. steeps in experiments during the three seasons 1932–4 and the results obtained are given in Table I.

Table I

Comparison of mercuric chloride and formaldehyde steeps

Year and variety			Percentage of scabbed tubers by weight					
			No treatment		Formaldehyde (1 : 240) 90-min. steep		0·1 % mercuric chloride 90-min. steep	
				Average		Average		Average
1932	Arran Crest		45·3	45·3	28·1	28·1	17·4	17·4
1933	Kerr's Pink		43·3	43·3	23·1	23·1	0·0	0·0
1933	Up-to-Date	(*a*)	57·6	60·3	9·8	5·9	0·0	0·3
		(*b*)	63·0		6·1		0·0	
		(*c*)	60·3		1·8		0·8	
1934	Arran Consul	(*a*)	85·7	85·2	35·8	51·4	8·4	23·6
		(*b*)	84·7		67·0		38·7	
1934	Up-to-Date	(*a*)	69·5	62·7	18·6	17·4	3·0	3·7
		(*b*)	59·4		18·8		4·5	
		(*c*)	59·1		14·7		3·6	
	Average		62·8		22·4		7·6	

These results indicate that the planting of scabbed non-disinfected seed potatoes in land which has not borne a potato crop for a large number of years may give rise to a very high percentage of scab in the resultant crop. The disinfection of the seed before planting by either the formaldehyde or mercuric chloride steep methods gave a marked reduction in the incidence of the disease. Mercuric chloride, however, was much more effective than formaldehyde, and, as this point first became evident in 1932, this method was included and was regarded as a standard for comparison in all subsequent experiments to test the efficiency of other fungicides.

Mercuric chloride as an instantaneous dip

The instantaneous dip method of tuber disinfection entails the immersion of the tubers in the fungicide for a period of ½–1 min. and has the pronounced advantage of reducing to a minimum the time required for the actual treatment, a factor of primary importance to the farmer. During 1934 and 1935 mercuric chloride was tested as an instantaneous dip at concentrations varying from 0·1 to 2·0 per cent and the results obtained are shown in Table II. At concentrations from 0·5 to 2·0 per cent the control of scab was excellent and practically as good as that obtained by the steep for 90 min. in a 0·1 per cent solution. 0·25 per cent

Table II

Comparison of instantaneous dips in mercuric chloride with the 90-min. steep

			Percentage of scabbed tubers by weight					
			1934 Majestic		1935 Arran Consul		1935 Kerr's Pink	
				Average		Average		Average
No treatment:		(*a*)	81·5	82·6	71·3	77·0	69·6	61·0
		(*b*)	83·6		75·1		52·9	
		(*c*)	—		76·2		66·4	
		(*d*)	—		85·5		54·9	
0·1 % mercuric chloride 90-min. steep:		(*a*)	5·3	8·0	15·9	15·0	11·4	11·2
		(*b*)	10·7		14·0		11·0	
Instantaneous dip in mercuric chloride:	0·1 %	(*a*)	27·1	36·8	54·3	48·2	23·7	22·7
		(*b*)	46·5		42·0		21·6	
	0·25 %	(*a*)	—	—	17·3	23·5	12·9	13·6
		(*b*)	—		29·6		14·3	
	0·5 %	(*a*)	1·1	1·5	10·9	15·4	16·3	16·3
		(*b*)	1·8		19·9		16·3	
	1·0 %	(*a*)	9·6	6·3	12·0	11·4	12·9	15·3
		(*b*)	2·9		10·8		17·6	
	2·0 %	(*a*)	—	—	6·5	8·3	7·4	13·4
		(*b*)	—		10·0		19·4	

gave reasonably good control, but the 0·1 per cent instantaneous dip was not satisfactory. The 2·0 and 1·0 per cent dips caused a marked depression in foliage growth, but this depression disappeared about 12 weeks after planting. The effect of treatment on growth and on the yield is discussed in a later section of the paper.

Mercuric chloride v. *copper sulphate and Burgundy mixture*

The Northern Ireland farmer is well acquainted with the preparation of Burgundy mixture for the control of potato blight, and as copper sulphate is easily procured in all districts these compounds were tested as tuber disinfectants. Copper sulphate was employed as a 90-min. steep and also at various concentrations as an instantaneous dip. 2 per cent Burgundy mixture (8 : 10 : 40) and 5 per cent Burgundy mixture (20 : 25 : 40) were used as instantaneous dips. The results are shown in Table III.

It will be seen that all the copper sulphate and Burgundy mixture treatments gave an appreciable reduction in the incidence of scab. None of the treatments, however, proved so effective as mercuric chloride,

Table III

Comparison of mercuric chloride and copper fungicides

			Percentage of scabbed tubers by weight					
			1934 Majestic		1935 Arran Consul		1935 Kerr's Pink	
				Average		Average		Average
No treatment:		(a)	81·5	82·6	79·1	80·1	52·8	54·0
		(b)	83·6		72·8		45·1	
		(c)	—		82·8		63·7	
		(d)	—		85·5		54·4	
0·1 % mercuric chloride 90-min. steep:		(a)	5·3	8·0	26·2	24·4	16·1	12·5
		(b)	10·7		22·6		8·9	
0·1 % copper sulphate 90-min. steep:		(a)	—	—	26·3	32·8	25·7	24·5
		(b)	—		39·3		23·3	
Instantaneous dip in copper sulphate:	0·25 %	(a)	—	—	31·5	42·9	21·2	34·1
		(b)	—		54·2		46·9	
	0·5 %	(a)	—	—	33·5	35·0	29·4	29·0
		(b)	—		36·4		28·6	
	1·0 %	(a)	—	—	32·6	38·3	16·3	19·2
		(b)	—		43·9		22·1	
	2·0 %	(a)	—	—	40·1	34·6	24·1	22·5
		(b)	—		29·0		20·9	
	4·0 %	(a)	—	—	31·0	29·0	18·8	18·2
		(b)	—		27·0		17·6	
Instantaneous dip in Burgundy mixture:	2·0 %	(a)	29·4	26·4	36·4	36·0	18·8	17·1
		(b)	23·3		35·6		15·3	
	5·0 %	(a)	17·1	16·0	39·5	32·3	15·1	15·1
		(b)	14·9		25·0		15·1	

while the higher concentrations of copper sulphate and 5·0 per cent Burgundy mixture had the further disadvantage of causing a severe depression in growth. In the case of the experiment with Arran Consul, the high incidence of scab throughout may be attributed to infection from the soil. The site of this experiment was adjacent to land which had borne a scabbed crop in the previous season, and infection of the soil was presumably brought about during the process of cultivation.

Mercuric chloride v. *organic mercurials*

During the last decade numerous organic mercury compounds have been recommended as short-period steeps or as instantaneous dips for the control of common scab and *C. Solani*. Proprietary materials of this type have been used by American workers, who have found some of them to be more effective than others; in some cases they have given as good results as the recognized standard mercuric chloride and formaldehyde treatments. At present a 15-min. steep in a solution of an organic mercurial is commonly practised in Holland for the control of *C. Solani*.

Table IV

Comparison of mercuric chloride steep and organic mercurial steeps

		Percentage of scabbed tubers by weight							
		1933				1934			
		Up-to-Date		Up-to-Date Stock A		Up-to-Date Stock B		Arran Victory	
			Average		Average		Average		Average
No treatment:	(*a*)	57·6	60·3	69·5	62·7	63·8	48·0	61·2	65·7
	(*b*)	63·0		59·4		32·1		63·1	
	(*c*)	60·3		59·1		—		69·7	
	(*d*)	—		—		—		68·5	
0·1 % mercuric chloride 90-min. steep:	(*a*)	0·0	0·3	3·0	3·7	3·7	6·0	11·8	7·8
	(*b*)	0·0		4·5		8·2		9·2	
	(*c*)	0·8		3·6		—		5·5	
	(*d*)	—		—		—		4·8	
Organic mercurial A:									
0·15 % 90-min. steep:	(*a*)	—	—	—	—	18·4	15·1	4·6	5·2
	(*b*)	—		—		11·8		3·5	
	(*c*)	—		—		—		2·7	
	(*d*)	—		—		—		10·0	
0·15 % 15-min. steep:	(*a*)	—	—	11·4	13·1	21·6	26·6	15·2	18·7
	(*b*)	—		9·0		31·6		13·6	
	(*c*)	—		19·0		—		30·8	
	(*d*)	—		—		—		15·3	
0·25 % 15-min. steep:	(*a*)	0·4	1·7	—	—	—	—	—	—
	(*b*)	0·9		—		—		—	
	(*c*)	3·7		—		—		—	

In the 1932 experiment with the variety Arran Crest a 15-min. steep in a 0·15 per cent solution of an organic mercurial was used, and the percentage of scab in the resultant crop was 6·2 per cent, a figure which compares very favourably with the 17·4 per cent given by the mercuric chloride steep. The same organic mercurial was tested in 1933 and 1934, 15- and 90-min. steeps being compared with the standard mercuric chloride steep (see Table IV). A satisfactory reduction in the incidence of scab was obtained, but on the whole the organic mercurial was not so efficient as mercuric chloride, and especially so when used as a 15-min. steep at 0·15 per cent. The 90-min. steep at the same concentration was more promising as was also the 15-min. steep at 0·25 per cent. The latter,

Table V

Comparison of steeps and instantaneous dips in organic mercurials

		Percentage of scabbed tubers by weight					
		1933 Up-to-Date		1934 Up-to-Date Stock A		1934 Up-to-Date Stock B	
			Average		Average		Average
No treatment:	(*a*)	57·6	60·3	58·3	55·0	62·5	54·8
	(*b*)	63·0		51·7		48·3	
	(*c*)	60·3		—		53·5	
0·1 % mercuric chloride 90-min. steep:	(*a*)	0·0	0·3	—	—	—	—
	(*b*)	0·0		—		—	
	(*c*)	0·8		—		—	
Steeps in organic mercurials A and B:							
A 0·15 % 15 min.	(*a*)	—	—	24·3	23·1	2·4	3·8
	(*b*)	—		21·9		7·8	
	(*c*)	—		—		1·3	
B 0·15 % 15 min.	(*a*)	—	—	47·3	34·9	19·2	13·0
	(*b*)	—		22·5		13·8	
	(*c*)	—		—		6·1	
A 0·25 % 15 min.	(*a*)	0·4	1·7	—	—	—	—
	(*b*)	0·9		—		—	
	(*c*)	3·7		—		—	
Instantaneous dips in organic mercurials A and B:							
A 0·5 %	(*a*)	—	—	18·5	16·5	3·8	4·1
	(*b*)	—		14·5		3·3	
	(*c*)	—		—		5·2	
B 0·5 %	(*a*)	—	—	36·2	27·6	25·0	16·9
	(*b*)	—		18·9		16·1	
	(*c*)	—		—		9·6	
A 1·5 %	(*a*)	0·5	1·2	9·1	7·7	0·0	1·4
	(*b*)	2·0		6·2		2·8	
	(*c*)	1·0		—		1·4	
B 1·5 %	(*a*)	3·6	1·3	18·4	18·9	3·8	3·5
	(*b*)	0·0		19·3		2·9	
	(*c*)	0·2		—		3·8	

however, was used only in one season, 1933, and from these results alone no definite conclusions can be drawn.

Further experiments to compare instantaneous dipping and steeping in solutions of organic mercurials were carried out, and the results obtained are shown in Table V. The mercuric chloride steep was included in the 1933 experiment only. Two proprietary organic mercurials (A and B) were used and proved to be more effective as instantaneous dips at strengths of 0·5 and 1·5 per cent respectively than when used as 15-min. steeps at lower concentrations. Material A used as a dip at 0·5 per cent was as efficient as B used at 1·5 per cent, while the amount of scab control in each case was entirely satisfactory.

The use of organic mercurial A as an instantaneous dip at various concentrations was also compared with the mercuric chloride steep. This material was recommended by the manufacturers to be used as a 15-min. steep at 0·15 per cent and the following concentrations, 0·05, 0·15, 0·45, 1·35 and 4·05 per cent, were used for the work. From the results given in Table VI it will be seen that the incidence of scab varied inversely with the concentration of the fungicide used. It must, however, be noted that severe growth depression in the crop resulted from the use of a concentration of 4·05 per cent.

Table VI

Comparison of mercuric chloride steep and instantaneous dips in an organic mercurial

			Percentage of scabbed tubers by weight			
			1934 Arran Victory		1934 Up-to-Date	
				Average		Average
No treatment:		(*a*)	62·2	59·4	52·2	50·4
		(*b*)	56·5		48·5	
0·1 % mercuric chloride 90-min. steep:		(*a*)	8·0	5·3	7·2	6·2
		(*b*)	2·5		5·1	
Instantaneous dip in organic mercurial A:	0·05 %	(*a*)	55·0	42·8	36·8	41·6
		(*b*)	30·6		46·3	
	0·15 %	(*a*)	28·4	28·3	33·0	29·3
		(*b*)	28·2		25·5	
	0·45 %	(*a*)	18·3	20·4	16·5	16·1
		(*b*)	22·5		15·6	
	1·35 %	(*a*)	3·4	5·4	9·1	8·3
		(*b*)	7·3		7·4	
	4·05 %	(*a*)	2·1	1·8	1·4	1·5
		(*b*)	1·5		1·6	

In 1935 materials A and B were again used as instantaneous dips at concentrations ranging from 0·5 to 3·0 per cent, the results obtained being shown in Table VII. The use of both materials gave a very appreciable reduction in the incidence of scab, which was especially marked at the higher concentrations employed. When used at the higher concentrations material B caused no growth depression, but A caused depression at strengths of 2·0 and 3·0 per cent.

Table VII

Comparison of mercuric chloride and organic mercurials

			Percentage of scabbed tubers by weight 1935 Kerr's Pink	
				Average
No treatment:		(*a*)	46·7	49·9
		(*b*)	50·3	
		(*c*)	47·1	
		(*d*)	55·3	
0·1 % mercuric chloride 90-min. steep:		(*a*)	3·5	6·9
		(*b*)	10·3	
Instantaneous dip in organic mercurial A:	0·5 %	(*a*)	9·8	13·2
		(*b*)	16·5	
	1·0 %	(*a*)	4·7	8·7
		(*b*)	12·6	
	1·5 %	(*a*)	4·6	5·7
		(*b*)	6·7	
	2·0 %	(*a*)	6·5	5·6
		(*b*)	4·7	
	3·0 %	(*a*)	7·8	5·5
		(*b*)	3·2	
Instantaneous dip in organic mercurial B:	0·5 %	(*a*)	15·7	17·6
		(*b*)	19·4	
	1·0 %	(*a*)	11·6	12·7
		(*b*)	13·7	
	1·5 %	(*a*)	10·5	8·6
		(*b*)	6·7	
	2·0 %	(*a*)	11·5	12·3
		(*b*)	13·0	
	3·0 %	(*a*)	9·3	7·8
		(*b*)	6·3	

III. LARGE-SCALE EXPERIMENTS

General

The results of the 1932 and the 1933 experiments at Hillsborough indicated that a large reduction in the incidence of scab could be obtained by the disinfection of the seed tubers before planting. These experiments, however, were confined to one centre and to land which had not borne a potato crop for many years. Hence it became desirable that experiments should be carried out at other centres, and that the efficiency of tuber disinfection should be tested in the case of a potato crop taking its place in the rotation or rotations practised by the farmer.

In 1934 and 1935 experiments were laid down on farms in County Down and County Armagh where scab was known to be prevalent and had in previous years rendered a large portion of the crop unmarketable. Three methods of tuber disinfection were employed, viz. 90-min. steep in 0·1 per cent mercuric chloride and instantaneous dips in organic mercurials A and B. In each case the potatoes were disinfected about 4 weeks before planting. The plots were randomized and duplicated, a plot consisting of four drills each 20 yards long (2 sq. perches). The experiments were laid down in the midst of the farmers' own crops. The manurial treatment was the same at most of the centres and was that commonly practised, i.e. 15 tons farmyard manure, 1 cwt. sulphate of ammonia, 4 cwt. superphosphate and 1 cwt. muriate of potash per statute acre. At harvest the results were taken from the two centre drills of each plot.

The 1934 *experiments*

In 1934 experiments were carried out at four centres. Organic mercurials A and B were used at concentrations of 0·5 and 1·5 per cent respectively. At three centres severely scabbed seed was used, while at the other centre (centre III—see Table VIII) only a proportion of the seed was slightly affected with scab. The results are given in Table VIII. These show that excellent control of scab was obtained at every centre by tuber disinfection. The mercuric chloride steep gave slightly better control than the organic mercurials, but, from a practical point of view, the control obtained by the latter was entirely satisfactory. At three of the four centres an interval of 6 years intervened between the planting of successive potato crops, while at the fourth centre the interval was only 1 year when the land was cropped with oats.

None of the experiments was conducted on land which had borne an infected potato crop in the previous season, but a trial was carried out at Hillsborough in which disinfected seed was planted in land which had borne a scabbed crop in 1933. The variety used was Up-to-Date, the seed being selected as free from scab. The method of disinfection employed was an instantaneous dip in 1·5 per cent organic mercurial B. The results, which were as follows, indicate that no control of scab was obtained:

		No treatment	Instantaneous dip in 1·5 % organic mercurial B
Percentage of scabbed tubers by weight:	(*a*)	34·7	37·7
	(*b*)	33·8	39·6
Average		34·3	38·7

This result, considered in conjunction with those of the county experiments, suggests that satisfactory control of scab may be obtained by the disinfection of seed tubers before planting provided that an interval of one or more years has elapsed between the taking of successive potato crops, but that no control is likely to be obtained in the case of potatoes grown on land which has borne a scabbed crop in the previous season. There was, however, only one case (centre IV) where a 1-year interval intervened between successive potato crops, and, from the results of this experiment alone, it is not possible to draw any definite conclusions as to the efficiency of tuber disinfection in the case of such a short rotation.

The 1935 *experiments*

The 1935 experiments were designed with the object of obtaining more information on the relationship between crop rotation and the control of scab obtained by tuber disinfection. Nine experiments were carried out, the intervals between successive potato crops being as follows:

(1) No interval (i.e. scabbed crop produced in 1934): 3 centres.
(2) 1-year ,, (,, ,, 1933): 1 centre.
(3) 2-year ,, (,, ,, 1932): 1 centre.
(4) 6-year ,, (,, ,, 1928): 4 centres.

Where no interval or only 1 year had elapsed, two types of seed were used, viz. scabbed seed (tubers selected as severely affected with scab) and clean seed (tubers selected by the farmer as free from scab: about 20 per cent of these had one or two small scab lesions per tuber). In cases where no interval elapsed between the taking of successive potato crops only the mercuric chloride steep method of disinfection was employed, while in the others instantaneous dips in organic mercurials A and B were used in addition. Organic mercurial B was used at 1·5 per cent as in 1934, but organic mercurial A was used at 1·0 per cent instead of 0·5 per cent. This change in concentration was made on the grounds of the results from the 1934 Hillsborough experiments which have already been described.

The results obtained are given below and are considered according to the interval elapsing between successive crops.

Table VIII

Results of large-scale experiments—1934

Land last cropped with potatoes	Centre	Variety		Percentage of scabbed tubers by weight							
				No treatment		Mercuric chloride steep		Organic mercurial A dip		Organic mercurial B dip	
					Av.		Av.		Av.		Av.
1927	I	Arran	(*a*)	80·1	80·9	2·5	3·2	12·7	12·3	8·8	10·9
		Consul	(*b*)	81·6		3·9		11·9		13·0	
	II*	Arran	(*a*)	71·5	66·5	2·9	3·6	11·3	9·0	4·9	7·0
		Consul	(*b*)	61·5		4·3		6·6		9·0	
	III	Arran	(*a*)	46·7	40·7	0·9	1·1	1·2	2·0	1·8	1·5
		Victory	(*b*)	34·6		1·2		2·8		1·1	
1932	IV†	Arran	(*a*)	69·1	67·9	10·8	9·1	2·9	7·1	8·0	8·6
		Victory	(*b*)	66·7		7·4		11·2		9·2	
		Average		64·0		4·2		7·6		7·0	

* At this centre, in addition to the standard manurial treatment, lime, at the rate of 2 tons per acre, was applied to the drills one week after planting.

† The incidence of scab was very severe at this centre in 1932 and, in the farmer's opinion, there was less than 30 cwt. of saleable potatoes from 2 acres.

(i) *No interval.*

The results which are set out in Table IX show that, while in some cases tuber disinfection reduced the incidence of scab, in others there was a greater proportion of scabbed tubers in the crops grown from

Table IX

Results from large-scale experiments, 1935—*no interval*

Centre	Variety	Type of seed planted		Percentage of scabbed tubers by weight			
				No treatment		Mercuric chloride steep	
					Average		Average
I	Kerr's Pink	Scabbed	(*a*)	82·0	83·5	24·3	31·3
			(*b*)	85·0		38·3	
		Clean	(*a*)	39·5	52·0	68·3	57·1
			(*b*)	64·5		45·9	
II	Kerr's Pink	Scabbed	(*a*)	66·1	66·8	85·3	61·9
			(*b*)	67·5		38·5	
		Clean	(*a*)	45·0	58·7	46·2	41·5
			(*b*)	72·4		36·8	
III	Majestic	Scabbed	(*a*)	58·3	63·3	42·2	48·3
			(*b*)	68·2		54·3	
		Clean	(*a*)	94·8	92·1	85·8	85·6
			(*b*)	89·3		85·3	
		Average		69·4		54·3	

disinfected seed. In one experiment (centre III) there was a significantly higher incidence of the disease in the crops raised from clean seed than from scabbed seed irrespective of tuber disinfection. It is therefore indicated that the source of infection was present in the soil, and the variation in attack which occurred from plot to plot and within each plot may be explained by the soil conditions either favouring or inhibiting the occurrence and spread of the disease. The results obtained from the Hillsborough experiment of 1934 are confirmed, and it is clearly indicated that tuber disinfection cannot be employed as a successful control measure when potatoes are grown in land which has produced a scabbed crop in the previous season.

(ii) *One-year interval* (potato crop last grown in 1933).

Only one centre could be obtained, and the results are given in Table X. The variety used was Arran Consul.

Table X

Result of large-scale experiments, 1935—*one-year interval*

		Percentage of scabbed tubers by weight							
Type of seed planted		No treatment		Mercuric chloride steep		Organic mercurial A dip		Organic mercurial B dip	
			Average		Average		Average		Average
Scabbed	(*a*)	88·5	86·7	81·4	69·7	28·8	28·8	27·8	45·8
	(*b*)	84·8		57·9		28·8		63·8	
Clean	(*a*)	88·4	76·6	43·4	33·7	—	—	—	—
	(*b*)	64·7		24·0		—		—	

The results are somewhat similar to those obtained where no interval intervened, but do not confirm those of the 1934 experiment where excellent control was obtained on land after a 1-year interval. It would appear that while in some cases tuber disinfection may secure good control, its use in such a short rotation cannot be expected to give consistently good results.

(iii) *Two-year interval* (potato crop last grown in 1932).

Again only one centre could be obtained; the variety used was Majestic and only one type of seed (scabbed) was planted. The results are given in Table XI.

These results suggest that there is more likelihood of tuber disinfection producing a considerable reduction in the incidence of scab where 2 years have elapsed between successive potato crops than in cases where the rotation is shorter. The somewhat inconsistent nature of the results obtained within some of the plots does, however, indicate that the production of satisfactory results in every case cannot be relied upon.

Table XI

Result of large-scale experiments, 1935—*two-year interval*

	Percentage of scabbed tubers by weight							
	No treatment		Mercuric chloride steep		Organic mercurial A dip		Organic mercurial B dip	
		Average		Average		Average		Average
(*a*)	93·8	93·8	25·2	24·6	52·3*	35·7	37·5*	25·4
(*b*)	93·8		24·0		19·0		13·3	

* The large percentage of scabbed tubers in these plots is due to the fact that in a portion of each plot, the portions being adjacent, very little control of scab was obtained, while in the remainder the measure of control was particularly good.

(iv) *Six-year interval* (potato crop last grown in 1928).

Experiments were laid down at four centres, and only scabbed seed was planted. The results are given in Table XII. Disinfection gave a large reduction in the incidence of scab at all four centres, although the percentage of scabbed tubers in some of the plots may appear excessive. The intensity of scab, however, on the tubers grown from disinfected seed was much less than that on the tubers from non-disinfected seed and, from a commercial standpoint, the measure of control obtained was estimated as being in the neighbourhood of 90 per cent. The instantaneous dips in organic mercurials proved as effective as the mercuric chloride steep.

Table XII

Results of large-scale experiments, 1935—*six-year interval*

			Percentage of scabbed tubers by weight							
			No treatment		Mercuric chloride steep		Organic mercurial A dip		Organic mercurial B dip	
Centre	Variety			Average		Average		Average		Average
I	Kerr's	(*a*)	60·4	64·0	18·6	13·9	16·5	13·8	19·9	13·5
	Pink	(*b*)	67·5		9·2		11·0		7·0	
II	Arran	(*a*)	88·0	86·1	22·6	23·8	18·0	23·1	28·3	27·7
	Consul	(*b*)	84·1		25·0		28·1		27·0	
III	President	(*a*)	74·1	79·6	31·9	33·7	16·0	26·1	27·8	36·2
		(*b*)	85·0		35·5		36·1		44·6	
IV	Kerr's	(*a*)	76·0	76·0	12·5	12·5	9·8	9·8	12·5	12·5
	Pink	(*b*)	—		—		—		—	
	Average		76·4		22·2		19·4		23·9	

These results, taken in conjunction with those of 1934, indicate that under Northern Ireland conditions when a 7-year rotation is practised, a very appreciable reduction of the incidence of common scab in the potato crop may be obtained by the disinfection of scabbed seed tubers before planting.

IV. THE EFFECT OF THE INTENSITY OF SEED TUBER INFECTION UPON THE INCIDENCE OF SCAB IN THE RESULTANT CROP

The scabbed seed employed in the experiments which have been described was so severely affected with the disease that it would not have been planted in ordinary farm practice. An examination, however, of the seed selected by farmers for their own plantings revealed that in many cases the incidence of scab was high and that it was not uncommon to have an average of two or three lesions per tuber. This observation raised the question as to whether the planting of seed slightly affected with scab, e.g. to the extent of one or two lesions per tuber, gives rise to as high an incidence of the disease in the resultant crop as the planting of very severely scabbed seed. This problem requires further investigation, but preliminary results obtained in 1935 are of sufficient interest as to warrant inclusion.

In the 1935 large-scale experiments with no interval both "clean" seed and scabbed seed were used. The "clean" seed was selected by the farmer as free from scab, but, on examination, it was found that a large percentage of the seed had a few small scab lesions per tuber. Samples of twenty tubers each of the "clean" and scabbed seed from centres I and III (see Table IX) were planted in land at Hillsborough which had not grown potatoes for many years. Ten tubers of each sample were disinfected by the mercuric chloride steep method, the remainder serving as controls. The results obtained are given in Table XIII. A large reduction in the incidence of scab resulted by tuber disinfection, indicating that the source of infection was seed-borne.

Table XIII

		Percentage of scabbed tubers by weight	
Variety	Type of seed planted	No treatment	0·1 % mercuric chloride 90-min. steep
Kerr's Pink	"Clean"	29·0	0·0
	Scabbed	52·8	5·8
Majestic	"Clean"	87·8	2·3
	Scabbed	94·0	15·0

Unfortunately no records were made of the actual amount of scab on each of the "clean" seed tubers planted. In no case, however, was the incidence more than a few small lesions per tuber, which were overlooked by the farmer when selecting the seed, while a few of the tubers were probably entirely free from scab. The incidence of the disease on the scabbed seed was severe, one-quarter to a half of the total surface of each tuber being affected.

The results obtained suggest that the planting of tubers slightly affected with common scab may give rise to a severe attack of scab in the resultant crop. The intensity of the disease in the case of the variety Majestic was particularly high and equally severe on the tubers from both "clean" and scabbed non-disinfected seed. In the case of Kerr's Pink the "clean" seed gave rise to a much smaller amount of scab than the scabbed seed. This may be explained by the fact that Kerr's Pink is not so susceptible to the disease as Majestic, and that the chances of the seed being entirely free from scab were greater in the case of the former than the latter.

V. THE EFFECT OF SEED TUBER DISINFECTION UPON THE GROWTH AND CROP YIELD

The effect of tuber disinfection upon the growth of the plant varied with the potato variety used, with different stocks of the same variety when grown at different centres, and also with the particular method of disinfection employed. In some cases disinfection gave a marked stimulus to growth whilst in others it caused a growth depression early in the season which disappeared as a rule from 10 to 12 weeks after planting. This growth depression occurred fairly consistently in the case of the variety Majestic, while with all the other varieties used the results obtained were more variable. The results suggest that a higher crop yield occurred in cases where a definite growth stimulus was noted, but the reverse did not hold, and in most cases where growth depression occurred early in the season there was no suggestion that the crop yield was lowered.

Of the treatments employed in the county experiments the mercuric chloride steep and the instantaneous dips in organic mercurial A caused *in most cases* a slight depression in growth. No depression was observed in the case of organic mercurial B.

At Hillsborough where copper sulphate, Burgundy mixture, mercuric chloride and organic mercurials were used as instantaneous dips at various concentrations the results indicated that these fungicides cannot be employed above certain concentrations without causing growth depression.

The relationship between seed treatment and growth depression has been investigated in the United States where experiments by White (28) suggest that no depression or "induced dormancy" occurs when the treatment is carried out in the autumn before the tubers have emerged from their dormant stage. This is a point which requires further investigation, for in all the experiments here described the tubers were treated in the spring and usually a few weeks before planting.

VI. Discussion

A survey of the literature dealing with the control of common scab of the potato indicates at once the difficulty of prescribing measures likely to be successful in every case. An attempt has been made to show this in the preliminary part of this paper where reference is made to the various practices which have been recommended from time to time, together with their attendant successes and failures. The ubiquity in the soil of scab-producing forms of *Actinomyces* is generally recognized, and the extent to which a potato crop grown in infected soil is likely to become affected with the disease has been considered by various workers to be due to the following factors acting either singly or in conjunction: (*a*) the amount of vegetable organic matter present in the soil, (*b*) the presence or absence of certain saprophytic species of *Actinomyces* and other micro-organisms, (*c*) the hydrogen-ion concentration of the soil, (*d*) temperature, and (*e*) moisture. The operation of these factors will explain the absence of the disease in potato crops grown in certain soils which have been exposed to infection, and also why in some cases "clean" crops may be produced irrespective of the amount of scab present on the seed tubers.

The results of these experiments, which deal with the possibility of controlling the disease by disinfection of affected seed, would appear to stress the importance of another factor which is the longevity of scab-producing forms of *Actinomyces* in Northern Ireland soils subject to normal crop rotation. The normal system of crop rotation followed on Northern Ireland farms occupies 7 years and is as follows: potatoes, oats, grass (4 years), oats. It has been shown that by the disinfection of scabbed seed tubers before planting a very satisfactory measure of control of the disease was obtained in soil which had produced a heavily infected crop 7 years previously and which was still capable of fostering a high incidence of the disease as occurred when non-disinfected seed tubers were used. The explanation of this would appear to lie in the fact that the system of crop rotation practised in Northern Ireland together with the interval which elapses between the taking of successive potato crops allows the scab organism to die out of the soil or encourages its loss of virulence to such an extent that a high incidence of the disease is only obtained when the soil is reinoculated by the planting of affected seed tubers. That seed tuber disinfection *cannot* be relied upon for the control of the disease in cases where the crop rotation is short is also shown, and this means that it is not a method suitable for adoption in normal garden practice where the soil is known to be infected. The results further indicate that the disinfection of seed tubers is not likely to produce consistently good results where an interval of less than about 4 years has elapsed between the growing of successive potato crops.

It is possible that the system of crop rotation employed is quite as important as the interval between successive crops. Tilford (32) records a case in Ohio where scab affected a potato crop taken in land put into cultivation for the first time after 40 years. Huisman (10) in Holland found scab prevalent in ploughed up grassland in spite of the fact that large quantities of green plant remains were present, while Lutman (11) in the U.S.A. records scab as being present in virgin soil where it was greatly increased by planting potatoes in the same land in the following year. Sanford (23) in Canada emphasizes the importance of such cultural practices as crop rotation and fallowing upon the incidence of the disease, while Berkner (3) in Germany points out that crop rotation exercises a decisive influence upon the development of common scab. He suggests a rotation for light dry soils which aims at maintaining a soil reaction not exceeding pH 6·0 and the growing of crops which make virtually equal demands upon the lime content. Goss (9) in U.S.A. suggests that cultivated crops or summer fallow the year preceding potatoes tend to raise the incidence of scab as compared with non-cultivated crops such as cereals. He remarks further that intervals of more than *four* years between potato crops tend to reduce scab attack, but that time alone is not sufficient to eliminate the disease or even to prevent serious loss.

When cultural practices are such as to diminish appreciably the pathogenicity of soil-borne *A. scabies* between the growing of successive potato crops, then the disinfection of affected seed tubers before planting must be regarded as a valuable control measure in that it prevents reinoculation of the soil through the medium of the seed.

VII. SUMMARY

1. This investigation deals with an attempt to determine the value of seed tuber disinfection as a method for controlling common scab (*Actinomyces scabies* (Thaxt.) Güss.) of the potato crop when tubers affected with the disease are used for planting.

2. A résumé is given of previous work by other investigators dealing with the control of this disease.

3. Under Northern Ireland conditions it has been found that a satisfactory control of the disease may be obtained by the disinfection of affected seed tubers before planting providing that a sufficient interval has elapsed between the growing of successive potato crops in the same land. After a 6-year interval, which is normal in Northern Ireland farm practice, satisfactory control was obtained in all cases, and it is suggested that a 4-year interval would probably be sufficient to ensure satisfactory results. Although good control has been obtained after the lapse of shorter intervals, such is not always the case, and the method cannot be relied upon with certainty. No control was obtained where no interval elapsed between the taking of successive potato crops.

4. Solutions of mercuric chloride, formaldehyde, copper sulphate, Burgundy mixture and proprietary organic mercury compounds employed in various ways were used in these experiments. The most satisfactory method for the farmer's use was found to consist of the instantaneous dipping of the tubers in solutions of proprietary organic mercurials.

5. The results obtained indicate that in some cases the planting of very slightly affected seed in land which has not carried potatoes for many years may result in a high incidence of the disease in the resultant crop.

6. A slight depression in growth was sometimes observed to result from the planting of disinfected tubers, and this was particularly noticeable in the case of the variety Majestic. After a period of from 10 to 12 weeks it disappeared and the crop grew out normally. In some cases tuber disinfection stimulated the growth of the plants. Some of the results obtained suggest an increase in tuber yield due to disinfection of the seed tubers, but the experiments were not laid down in such a way as to permit a statistical analysis of the yields being made.

VIII. Acknowledgements

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STUDIES IN BACTERIOSIS

XXIII. FURTHER STUDIES ON A BACTERIUM CAUSING FASCIATION OF SWEET PEAS

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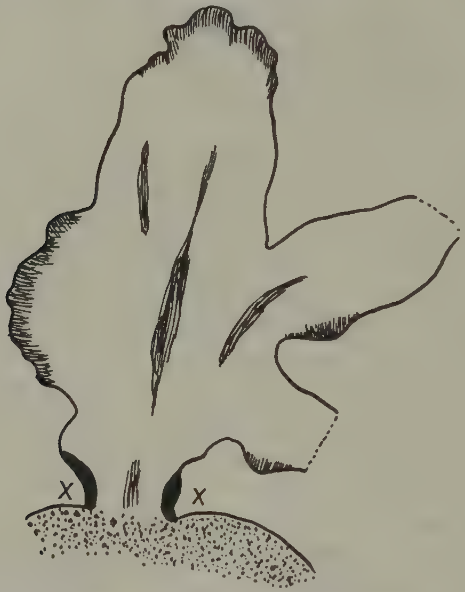
(With Plate XXXIII and 7 Text-figures)

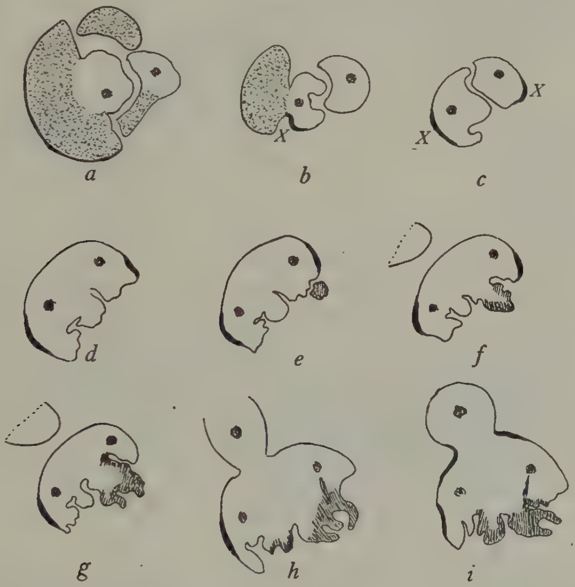
In a previous paper (1) an account was given of the isolation from fasciated sweet peas, leafy galls of chrysanthemum, carnation and *Schizanthus retusus*, and from "Cauliflower" strawberry plants of strains of a bacterium all of which produced severe fasciation when inoculated on to young sweet-pea seedlings. Since then strains of the same bacterium have been isolated from four other host plants, namely, from a leafy gall of *Nicotiana glutinosa*, from asparagus galls, from an abnormal growth on *Heuchera sanguinea* (Pl. XXXIII, fig. 1), closely resembling the "cauliflower" disease of strawberry plants, and from a large gall on a *Gladiolus* corm (Pl. XXXIII, fig. 2). These four new strains were all pathogenic to sweet-pea seedlings.

It was stated in the earlier paper that excellent infections had been obtained by growing sweet-pea seedlings in small glass bottles on sand inoculated with a culture of the bacterium. This method has been used to test the virulence of newly isolated strains and also to obtain fasciated seedlings for work on the histology of the disease, some account of which is given in this paper. For this work sweet-pea seeds were sterilized in one in a thousand mercuric chloride, thoroughly washed in sterile water and placed on moist filter paper in Petri dishes for 4 days, then dropped singly into the bottles, the sand in which was inoculated with a strain of the sweet-pea organism. At intervals seedlings showing positive infections were removed from the bottles, some being fixed for sectioning and others used for isolation experiments. The macroscopical examination of the infected seedlings showed that the abnormal growth occurred in the hypocotyl region. In some cases the plumule was only slightly affected, but usually it was stunted, often failing to grow more than an inch or so in height. At the point of attachment of the plumule to the cotyledons a number of secondary shoots would develop, these again being stunted, distorted and swollen, with fleshy gall-like masses of tissue at the base (Pl. XXXIII, fig. 3, *e* and *f*). Quite frequently the plumule failed to grow, but from the hypocotyl region two thick fleshy growths attached to a swollen mass of tissue developed (Pl. XXXIII, fig. 3, *a* and *d*). In other cases a single tumour-like mass was formed (Pl. XXXIII, fig. 3, *b* and *c*). The roots never showed any hypertrophy, and in mild cases they developed normally. In severely infected seedlings, such as those shown in Pl. XXXIII, fig. 3, the radicle soon ceased to grow, without, however, showing any sign of fasciation.



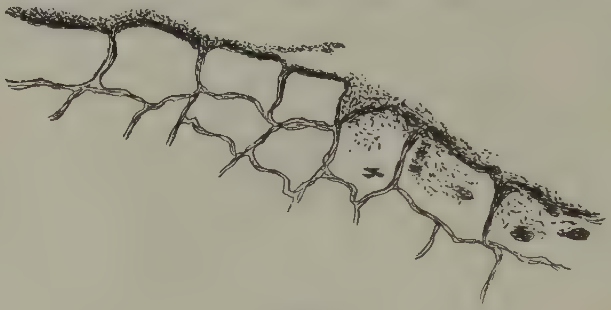
Text-fig. 1. Diagram of a section of a sweet-pea seedling cut 2 weeks after inoculation.

A series of microtome sections of the hypocotyl region of seedlings of the type shown in Pl. XXXIII, fig. 3, from a fortnight to 11 weeks after inoculation were made and stained with carbol thionin and orange G. The sections were cut as far as possible at right angles to the cotyledonary petioles, the first series passing through the cotyledons, the next through the petioles at their junction with the embryo, and the last series through the shoots. Text-fig. 1 is a diagram of part of a cotyledon (stippled) with the stalk and young shoot attached, cut in an oblique transverse direction, 2 weeks after inoculation. The pathogen appears to have no effect on the cotyledons; throughout the sections there is no indication of invasion of the cotyledonary tissue. The shoot is already showing definite hypertrophy, it is much swollen and irregular outgrowths have developed. The shading marks areas of small-celled, very actively dividing meristematic tissue. The interior consists of large-celled parenchyma with three groups of vascular tissue. Although groups of bacteria can be seen on the outside of the shoot there is no indication of their entry into the epidermal cells or of any damage to these cells. In the region of the cotyledonary petiole, however, the bacteria lie in dense zoogloeal masses against the epidermal walls. Many of the epidermal cells are filled with bacteria; some of the cells are disintegrated, and in others the cell walls are swollen, mucilaginous, and dead, as shown by deep staining with thionin blue. These necrotic areas are indicated in the diagram by the black lines marked *X*.

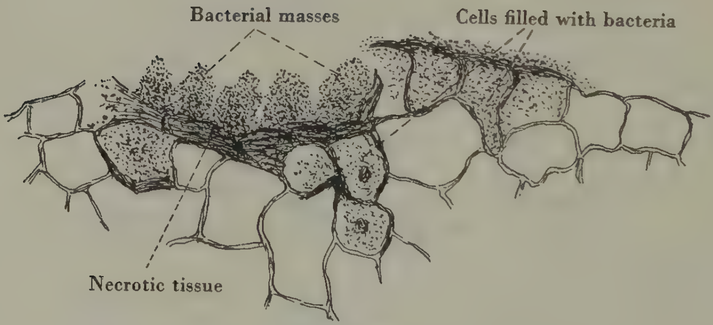


Text-fig. 2. Diagrams of a series of sections through the cotyledons, petioles and shoot of a seedling 3 weeks after inoculation.

Text-fig. 2 shows diagrams of a series of sections through the cotyledons, petioles and shoot of a seedling 3 weeks after inoculation. The stippled areas in sections *a* and *b* are cotyledonary tissue, and the two unshaded areas are the petioles, each with a vascular bundle in the centre. In section *b* the black line marked *X* indicates an area in which the epidermal cells are attacked by bacteria. In section *c* there is a necrotic epidermal area on both petioles which persists in the succeeding sections. Between these necrotic areas in sections *d* to *i* there is on one side the root tissue, where the outline is smooth and regular, and on the other side the shoot, with surface development of irregular outgrowths seen first in section *d* and becoming more and more marked in succeeding sections. The shading indicates areas of actively dividing cells. In this region groups of bacteria are found on the exterior, but they are not in contact with the epidermal walls and they apparently have no adverse effect on the tissue. Parts of the necrotic areas indicated by the black lines in Text-fig. 2 are shown in detail in Text-figs. 3 and 4. Text-fig. 3 shows a thick bacterial zoogloea in close contact with the outer cell walls of the epidermis; these walls are swollen and mucilaginous, but on the left the cells have not yet been invaded by bacteria. On the right the outer wall has broken down and bacteria are filling the cells. Text-fig. 4



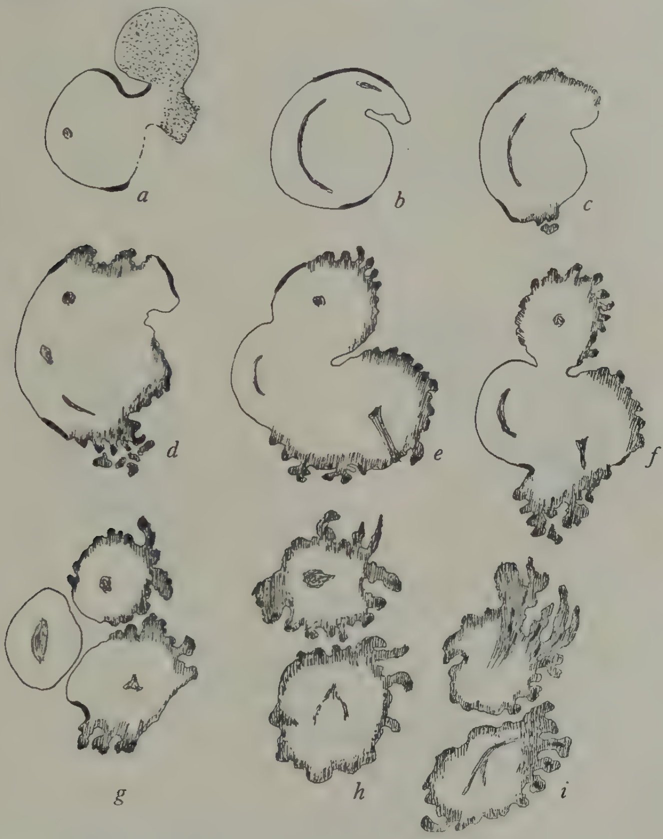
Text-fig. 3.



Text-fig. 4.

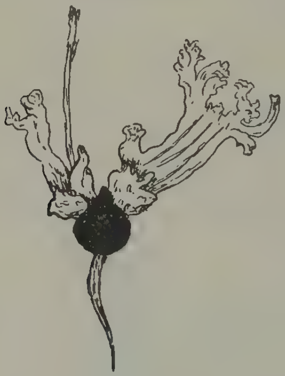
Text-figs. 3 and 4. Parts of the necrotic areas indicated by the black lines in Text-fig. 2. ×450.

shows a further development; the cell walls of the epidermis have become disintegrated, but the thick bacterial masses still roughly mark the outline of the interior of the cells which they filled. Below these are the swollen, dead remains of the inner walls of the epidermal cells, and one or two of the underlying cells have also been invaded.

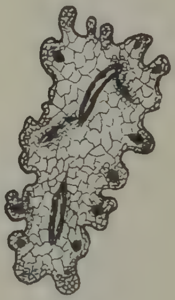


Text-fig. 5. Diagrams of a series of sections through a sweet-pea seedling 7 weeks after inoculation.

The diagrams in Text-fig. 5 show a further stage in the development of fasciation in a seedling 7 weeks old. In this seedling two bulbous fleshy outgrowths and a very short radicle had been produced from the embryo, similar to the seedling shown in Pl. XXXIII, fig. 3, *d*. In Text-fig. 5, section *a*, the stippled area indicates part of a cotyledon and the smooth outline on the left marks root tissue. Between the two on each side are areas (indicated by the black lines) where the epidermal cells are destroyed and filled with bacteria which have at this age penetrated into the intercellular spaces to a depth of five or six cells. In section *c* the irregular outline of the two shoots is apparent; this becomes more marked in succeeding sections, where the shaded areas show regions of actively dividing meristematic tissue. In section *g* the two shoots and the root have separated; the root section is normal, with a smooth even outline, in contrast with the two shoots, in which numerous small outgrowths have arisen from the irregular, indented surface tissue; sections *h* and *i* show the further developments of these outgrowths. At the time this seedling was fixed it had ceased growth. In natural conditions, in unsterilized soil, a growth of this type would probably never appear above ground. After the epidermal cells were destroyed the dead tissue would be attacked by saprophytic soil bacteria and the whole growth would quickly rot.



Text-fig. 6



Text-fig. 7

Text-fig. 6. Sweet-pea seedling 11 weeks after inoculation. Nat. size.
Text-fig. 7. Transverse section through the fasciated outgrowth shown in Text-fig. 6.

Sections of seedlings made 4, 5, 6 and 11 weeks after inoculation were similar in appearance to those already described. In all, necrotic areas filled with bacterial masses were present in the epidermal tissue of the cotyledonary petioles, and, in places, in the lower parts of the shoots. A drawing of the seedling sectioned 11 weeks after inoculation is shown in Text-fig. 6. The plumule was dead, and in addition to the usual swollen shoots there was one wide, flattened growth suggesting the anastomosis of several stems. Text-fig. 7 is a diagram of a transverse section through this growth. There are two central steles from which vascular bundles are sent out to each of the small outgrowths which have developed all round the exterior. The smaller outgrowths consist entirely of small-celled tissue; the larger have four or five irregular rows of small cells on the exterior, but the internal tissue is composed of large parenchymatous cells. There was no bacterial invasion of the tissue in any part of this fasciated shoot, and, although occasional small groups of bacteria were present on the outside, there was no sign of a bacterial zoogloea on the epidermis.

Throughout these series of sections appearances suggest that the bacteria are able to cause abnormal proliferation of tissue at some distance from their location either outside or inside the epidermal cells. Various attempts have been made to produce this proliferation by growing sweet-pea seedlings in sand watered with bacteria-free filtrates obtained either from extracts of fasciated shoots or from filtered cultures of the organism, but the experiments have given negative results.

The reisolation of the organism from inoculated sweet-pea seedlings similar to those used for sectioning confirmed the evidence obtained from the microscopical examination that the pathogen is mainly confined to the exterior of the tissue. The isolations were made from the basal swellings of seedlings from 3 to 7 weeks after inoculation in the following manner. The growth was dropped into sterile water in which it was left for 15 min. and one loopful of this water was plated on bouillon agar. The tissue was then transferred to one per thousand mercuric chloride for 5 min. and afterwards washed in sterile water. The inner tissue was scraped into sterile water, and after standing for a further 15 min. a loopful of this was plated on bouillon agar and in addition pieces of the tissue were embedded in the agar. The first isolations were made 3 weeks after inoculation of the seedlings, when a swollen hypertrophy had already developed in the hypocotyl region; further isolations were made from seedlings 4, 5, 6 and 7 weeks after inoculation. Table I shows the results of these inoculations.

Table I

Growth of the pathogen on isolation plates from inoculated sweet-pea seedlings

Inoculation period	No. of colonies on plates from surface washings	No. of colonies on plates from inner tissue
3 weeks (1)	Innumerable	15
(2)	,,	0
4 weeks (1)	,,	0
5 weeks (1)	,,	24
(2)	,,	0
6 weeks (1)	,,	10
(2)	,,	28
7 weeks (1)	,,	5
(2)	,,	70

It is evident from this table that nearly all the bacteria are destroyed by surface sterilization. The 7 weeks' seedling which yielded the greatest number of colonies (70) from the inner tissue was beginning to decay and, in addition to the seventy pathogenic colonies, twenty-seven colonies of saprophytic organisms were also present on the isolation plates.

In being confined mainly to the outer surface of the affected tissue this new pathogen resembles *Bact. tumefaciens.* Robinson & Walkden (2), working with the rough galls on *Chrysanthemum frutescens*, found that a mucilaginous film containing very large numbers of bacteria was invariably present on the surface of galls from 10 days to 2 months old. The sweet-pea organism forms a similar film on the exterior of the hypocotyl region of the seedlings. Further, Robinson & Walkden state that dipping the unbroken gall of *C. frutescens* into mercuric chloride solution for 10 sec. was sufficient to reduce the numbers of *Bact. tumefaciens* from 200 per plate to one, and in a series of isolation tests made with galls from 5 to 8 weeks old they found that merely washing the gall in running water for 4 hours removed nearly all the organisms. They conclude that "in the actively growing galls with rough surface on *Chrysanthemum frutescens*, there are enormous numbers of bacteria situated on the exterior of the gall, that a high percentage of these is *Bact. tumefaciens,* and that it is reasonable to assume that the presence of these progressively increasing numbers of *Bact. tumefaciens* on the exterior provides the progressive stimulus which leads to the continued growth of the gall". This assumption might equally well be made concerning the action of the sweet-pea organism.

SUMMARY

1. Strains of a bacterium causing fasciation of sweet peas have been isolated from galls on *Nicotiana glutinosa*, asparagus, *Heuchera sanguinea* and *Gladiolus* in addition to the host plants reported in a previous paper. These four new strains all produced fasciation on inoculation into sweet-pea seedlings.

2. Histological examination of sweet-pea seedlings 2–11 weeks after inoculation showed that abnormal growth occurred in the hypocotyl region. In certain areas bacterial zoogloea formed a film on the outside of the tissue, and some penetration of bacteria into the epidermal cells, and in later stages into the intercellular spaces, was observed.

3. Reisolation tests confirmed the evidence from the microscopical examination, that the pathogen is mainly confined to the exterior of the tissue.

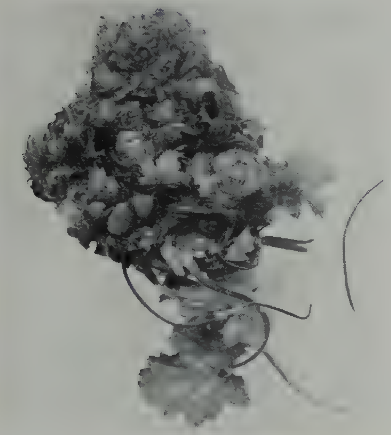


Fig. 1

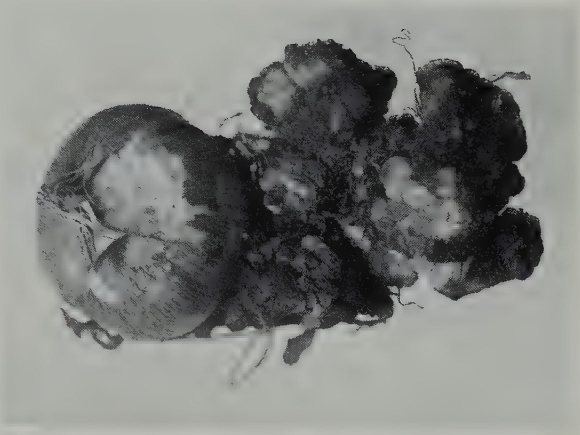


Fig. 2

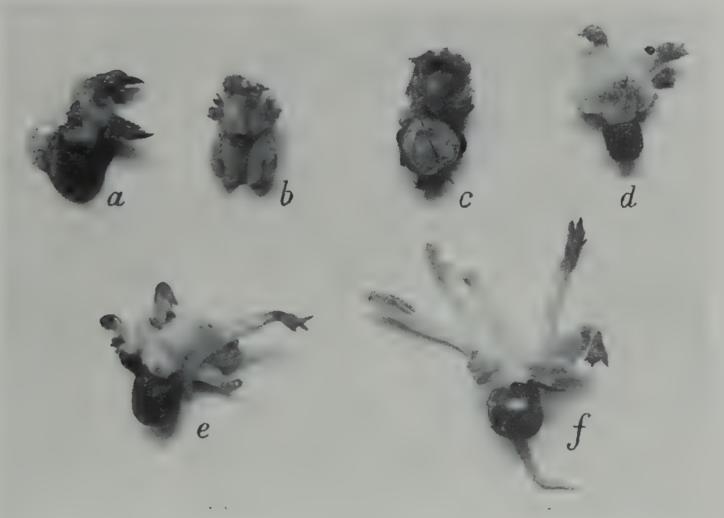


Fig. 3

LACEY.—Studies in Bacteriosis (pp. 743–751)

4. A comparison is made between the action of the sweet-pea organism and *Bact. tumefaciens* in causing abnormal plant growths.

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EXPLANATION OF PLATE XXXIII

Fig. 1. *Heuchera sanguinea* gall. Nat. size.

Fig. 2. *Gladiolus* corm gall. Nat. size.

Fig. 3. Types of fasciation produced in sweet-pea seedlings by inoculation of the sweet-pea organism. Nat. size.

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THE HISTOLOGY OF THE NECROTIC LESIONS INDUCED BY VIRUS DISEASES

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(With Plates XXXIV and XXXV)

Introduction

Many viruses produce widely differing symptoms in different hosts. Some, which generally result in a systemic infection may, in a few hosts, induce the production of local necrotic lesions. Tobacco mosaic disease and aucuba (or yellow) mosaic of tomato produce a local necrosis in *Nicotiana glutinosa* although cucumber mosaic and Hy. III disease cause mottling in this same host. To produce systemic infection, the virus spreads from the seat of inoculation throughout the tissues of the host and into the primary meristem where it interferes with the normal development of the plant tissues (6). In tobacco, some diseases (e.g. aucuba mosaic) may induce local necrotic lesions on the inoculated leaf but the disease later becomes systemic. When necrotic lesions are produced in *N. glutinosa* or in the bean the virus is confined to a relatively small number of cells surrounding the seat of inoculation. These cells die, necrosis becoming macroscopically visible about 2 days after inoculation. Such lesions have proved of considerable importance in this field of pathological research, as they provide a quantitative test for the viruses producing them. It seemed desirable, therefore, to discover the special peculiarities in the behaviour of the host which result in the isolation of the virus within local necrotic areas.

Material and methods

Juice was extracted from macerated leaves of tomato or tobacco plants infected with aucuba mosaic disease. A dilution of one part of this crude juice in two parts of water was used to inoculate the leaves of *N. glutinosa* plants. The juice was rubbed with the index finger on to one-half of each leaf, care being taken to injure nothing but the hairs. The unrubbed halves of the leaves were used as controls. Generally, the virus was rubbed on to the adaxial surface of the leaf. As the work developed, it became necessary to inoculate a few leaves by rubbing the lower surface. As controls, leaves were rubbed with healthy juice and others with water.

Small strips cut from each half of each leaf were later fixed. In the summer under good growing conditions when the lesions became macroscopically visible in less than 2 days, fixations were made at intervals of 12, 24, 48 and 72 hours after inoculation.

A large number of fixatives was tried and of these, Zenker's, Champy's, Regaud's and LaCour's fluids were selected for further work. Owing to the waxy nature of the leaves and to the hairs on the surface, difficulty was experienced in making the leaves sink into the fixing fluid. This was to some extent overcome by immersing them in Carnoy's fluid for about 1 min. prior to using the appropriate fixative. The fixing bottles were then placed under an exhaust pump until the material would sink in the fluid.

After fixing the material was washed in running water, dehydrated in ethyl alcohol, cleared in cedar wood oil and embedded in paraffin wax of melting-point 52° C. Sections were cut at a thickness of 6–15μ and were variously stained.

DESCRIPTION

The normal leaf

In transverse section the healthy leaf of *N. glutinosa* (Pl. XXXIV, fig. 1) shows a single row of closely packed, elongated, thin-walled palisade cells towards the upper surface. Below this are several layers of parenchymatous cells forming a meshwork with large intercellular spaces. Each surface is bounded by a highly cuticularized epidermis. The majority of the stomata are on the lower surface of the leaf where they are in direct connexion with the large air spaces of the spongy parenchyma. The surface of the leaf is protected by hairs which appear to be more numerous on the upper than on the lower side. Each hair grows out from an enlarged epidermal cell and consists of about four elongated cells tapering towards the apex. The walls of the hairs are strongly cuticularized.

The development of the lesions

Under good growing conditions lesions become evident on the dorsal surface of the leaves about 48 hours after inoculation. Minute, slightly sunken, dried patches of epidermis are visible on the lower surface a few hours previously. The spots do not all develop simultaneously, their numbers being found to increase over several days. At first, the lesions appear as small, colourless shiny patches. For several days they increase in size, at the same time becoming dry and assuming a brown coloration.

The first reaction to the virus may become microscopically visible after 12 hours but more usually no abnormality is apparent until 24 hours after inoculation. The first visible evidence of infection is the appearance of a strip of dark staining material between certain of the cell walls. Occasionally, this band may first appear between the palisade cells, spreading rapidly downwards from towards the upper to the lower side of the leaf. More frequently the band is first formed between the cells of the lower epidermis and those of the spongy parenchyma (Pl. XXXIV, figs. 2 and 4).

The virus enters a leaf through hairs which were broken by rubbing (8). However, lesions do not always develop immediately below a broken hair. Presumably the virus may travel a short distance across, as well as through the leaf, before any reaction is shown. The dark staining disc is at first apparent over only two or three cells, but it rapidly spreads until it covers a hundred or more (Pl. XXXIV, fig. 4), completely cutting off the epidermis from the spongy parenchyma.

At the same time as this band is forming, mitosis occurs in the cells immediately within it. All stages in nuclear division from the prophase to the presence of two daughter nuclei within one cell were found (Pl. XXXV, figs. 1–4). Karyokinesis appeared to be normal, but no evidence of cytokinesis was found. At the telophase, no cell-wall formation was seen, and binucleate cells are of fairly frequent occurrence in the areas which are becoming necrotic. Mitosis usually occurs in the cells immediately within the epidermis (Pl. XXXIV, figs. 2 and 4 and Pl. XXXV, figs. 1–4) but it is sometimes more deep seated (Pl. XXXIV, figs. 6 and 7).

The necrotic disc has now isolated the epidermis, and the cells, being cut off from their supplies of nutriment, are drying up. The band begins to branch upwards towards the upper surface of the leaf (Pl. XXXIV, figs. 5–7). It ramifies between the cells of the parenchyma, ultimately penetrating through to the upper surface. As each cell is isolated it dies and dries out. Finally the whole lesion becomes dry and consists essentially of a meshwork of the dark staining necrotic material (Pl. XXXV, fig. 7).

That the changes described are all due entirely to the interaction of the virus and the host is shown by the examination of the control material. When leaves were rubbed with water or with healthy plant juice, the only abnormalities found were broken hairs, there was no stimulation to mitosis nor was there any tendency to the formation of the dark staining disc.

Experiments were made to determine the nature of the necrotic material. The peculiarity of its first appearance being usually towards the lower surface where there are large intercellular spaces and many stomata suggested that it might be a product of oxidation. Two experiments were therefore made.

Three leaves were taken on each of six plants. Half of each leaf was rubbed with virus extract in the usual way on the upper surface. The other half was rubbed on the lower surface with the same extract. Both sets of lesions developed simultaneously. It was evident that placing the virus near the seat of the first visible reaction in no way hastened that reaction. There was, however, a difference in the numbers of lesions. A total of 2872 developed on the halves rubbed on the upper side, whilst rubbing the lower side resulted in only 1526 spots. This is probably due to there being fewer hairs on the lower surface of the plant. It also refutes the suggestion (2) that the virus enters through the stomata. As there are more stomata on the lower than on the upper surface, if the virus entered through them, the greater number of lesions would be expected to develop on the ventral surface.

Virus extract was then rubbed over the whole dorsal surface of some leaves. Immediately vaseline was smeared over half of the lower surface of each inoculated leaf. No difference could be discerned in the development of the lesions on the two halves of the leaves. These experiments indicated that the larger supply of air available near the lower surface is not essential to the rapid formation of the necrotic material.

This material is yellow in colour and on casual examination appears to have an affinity for basic dyes. Critical examination of stained preparations shows it to consist of large numbers of slightly elongated minute particles embedded in a matrix (Pl. XXXV, figs. 5 and 6). The particles are basophilic but the matrix stains with acid dyes.

As the substance appears first within the cell wall, it was thought that it might be of a pectic nature. Microchemical tests with ruthenium red and methylene blue on hand sections of fresh material and on microtome sections of fixed material lent no support to this view, nor did the substance react to treatment with pectinase. It proved to be insoluble in strong concentrated acids and in caustic alkalis. No positive reactions could be obtained with any of the more common reagents such as

Millon's, Sudan III, chlorzinc iodide, phloroglucin, ferric chloride, resorcin blue, etc.

DISCUSSION

That all living cells are capable of division has for some time been realized (1, 4). After wounding, a cambium may be formed across the cells of the most varied tissues in a layer near the surface of the wound. Meristems are formed also in response to fungal or bacterial infection.

In *Nicotiana glutinosa*, cell division normally ceases when the leaf is less than 1 cm. in length. Cell differentiation then commences and further growth is due entirely to enlargement of the cells. The leaves at the time of inoculation are about 10 cm. long and cell division has ceased 2 or 3 weeks previously. At the time of inoculation the spongy parenchyma cells are large and vacuolate and the nuclei are relatively small. It is obvious that without some external stimulus, no further division would occur. The necessary stimulus is supplied by rubbing the surface of the leaves with virus. That the mitosis is not a wound reaction is shown by the examination of leaves rubbed with water or with juice extracted from healthy plants. Such leaves fail to reveal any nuclear divisions, suggesting that the stimulation is provided directly or indirectly by the virus. Possibly this unusual mitosis is an attempt by the host to isolate the etiological agent of the virus disease by hyperplastic growth, and the cells are overcome by necrosis before new growth can occur. Or, possibly, the virus causes chemical reactions in the cells which produce the necrotic material and the products of reaction stimulate the nuclei. It is known that small doses of toxic substances often cause excitation of the cell contents (7).

It seems most probable that the mitosis is directly due to stimulation set up by the virus. In a number of solanaceous hosts infected with systemic diseases it has been found that the first symptom of the virus to become microscopically visible is a stimulation of the cytoplasm (5, 7). In these cases nuclear division is not found. The woody disease of *Passiflora edulis* caused by a virus (1) results in abnormal formations through hypertrophy of the pericarp. In sugar beet infected with curly-top disease, cells adjacent to lesions are stimulated to growth and division (3). The occurrence of karyokinesis as the lesions develop in *Nicotiana glutinosa* seems to be a parallel case of direct response to the stimulus of the virus.

The necrotic band formed between the cells may be due to the increased and abnormal metabolism causing exudation of waste products from the cells. The necrotic material may cause suffocation of the tissues within it by preventing free interchange of gases with the atmosphere and so preventing the continuation of cell division, or the necrotic materials may be toxic to the cells.

When the virus first enters the leaf it obviously multiplies and travels rapidly through the cells. The response of the host is even more rapid, free interchange of materials between the infected cells and the surrounding tissues is soon prevented by the necrotic barrier. The virus is isolated within a relatively small area of the leaf within about 48 hours after infection.

Summary

About 12 hours after a leaf of *Nicotiana glutinosa* is inoculated with aucuba mosaic disease a band of necrotic material begins to form within the cell wall, usually between the lower epidermis and the spongy parenchyma. This band extends both laterally and towards the upper side of the leaf. At the same time, nuclear division is observed in the spongy parenchyma cells, but karyokinesis is not followed by cell division. As the necrotic meshwork extends the cells within it die and dry out. After about 3 days, the lesion consists of a meshwork of this necrotic material. The cells are all dead and the virus is isolated within the necrotic area, all interchange between the infected and healthy parts of the leaf having been prevented.

I am indebted to Fraulein Lina Cunow for assistance in making the preparations used in this study.

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EXPLANATION OF PLATES XXXIV AND XXXV

All photomicrographs were taken with a Leitz "Makam" camera. Leitz objectives 3 *b* or 6 L achromatic or 2 mm. apochromatic (N.A. 1·4) were used in combination with Leitz periplanatic oculars × 10 or × 6.

A Leitz "Monla" lamp, suitably screened with Wratten colour filters, was employed as the source of illumination.

The fixative and the stain used in making the preparation, together with the colour filter used to take the photograph, is given in brackets after the description of each figure.

PLATE XXXIV

Fig. 1. Transverse section of normal leaf of *Nicotiana glutinosa* showing single layer of palisade tissue and several layers of spongy parenchyma. The leaf is surrounded by a cuticularized epidermis. × 280. (Lacour. Safranin-light green. Orange 22.)

Fig. 2. 24 hours after inoculation. Transverse section of leaf showing early formation of necrotic material within the lower epidermis. Mitosis is occurring in a cell immediately within the necrotic band. × 280. (Zenker. Crystal violet-erythrosin. Green 58.)

Fig. 3. 24 hours after inoculation. Transverse section of leaf showing early formation of necrotic material. × 280. (Zenker. Safranin-light green. Green 66 and blue 45 A.)

Fig. 4. 48 hours after inoculation. The necrotic disc is extending laterally. Mitosis occurs in the cells immediately within the band. × 140. (Zenker. Safranin-light green. 66 and 45 A.)

Fig. 5. 48 hours after inoculation. The necrotic material extends upwards through the leaf. × 140. (Zenker. Safranin-light green. 66 and 45 A.)

Fig. 6. As fig. 5. Mitosis occurs in more deep-seated cells. × 450. (Zenker. Safranin-light green. 66 and 45 A.)

Fig. 7. 48 hours after inoculation. The necrotic material has spread almost to the upper surface of the leaf. Mitosis occurs in the upper epidermis. × 450. (Zenker. Safranin-light green. 66 and 45 A.)

PLATE XXXV

Fig. 1. 48 hours after inoculation. Metaphase in a cell within the necrotic disc. × 900. (Zenker. Safranin-light green. 66 and 45 A.)

Fig. 2. 48 hours after inoculation. Anaphase in a cell within the necrotic band. × 900. (Zenker. Safranin-light green. 66 and 45 A.)

Fig. 3. 48 hours after inoculation. Telophase in a cell within the necrotic band. × 900 Zenker. Safranin-light green. 22.)

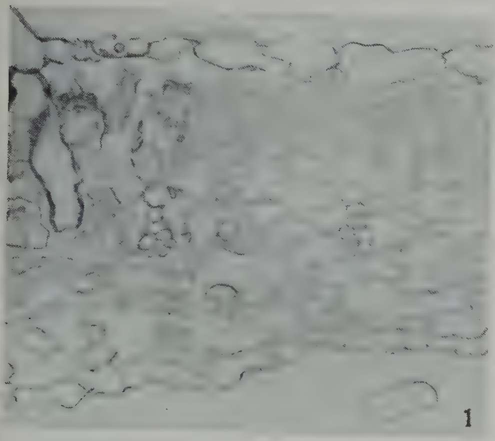
Fig. 4. 48 hours after inoculation. Two daughter nuclei within one cell within the necrotic disc. × 900. (Zenker. Safranin-light green. 66 and 45 A.)

Figs. 5 and 6. 48 hours after inoculation. High power photographs of the necrotic material show it to be of a particulate nature. × 900. (Zenker. Crystal violet-erythrosin. 22.)

Fig. 7. 72 hours after inoculation. Transverse section of leaf passing through a necrotic lesion. × 84. (Zenker. Safranin-light green. 66 and 45 A.)

(*Received* 18 *May* 1936)

PLATE XXXIV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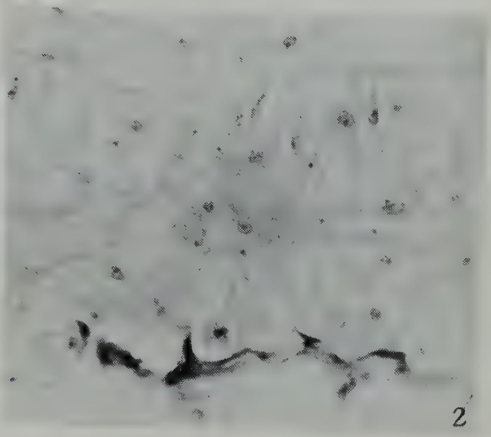
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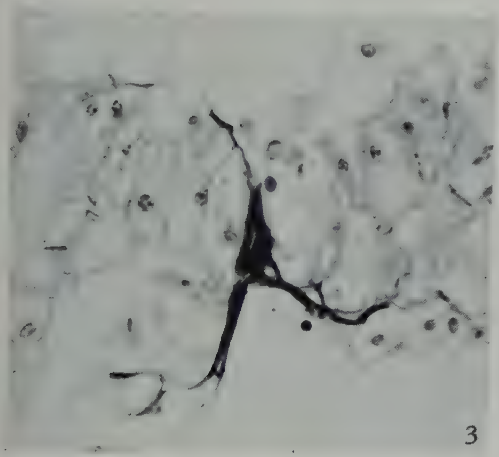
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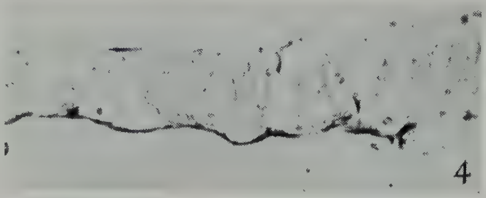
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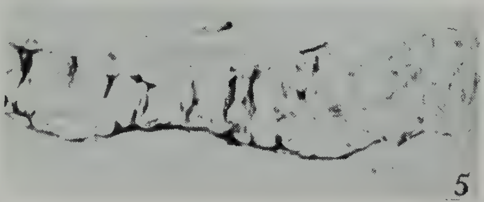
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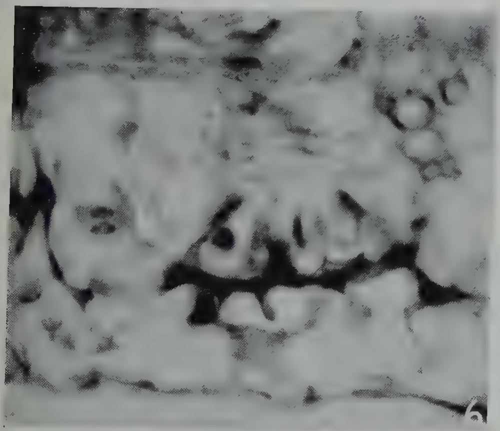
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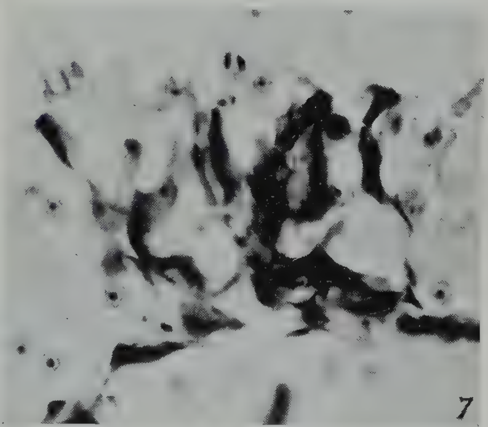
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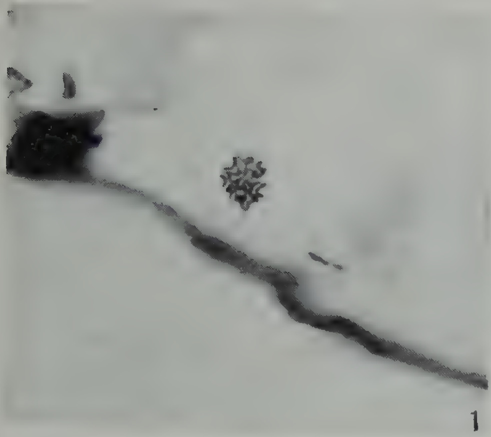
SHEFFIELD.—The Histology of the Necrotic Lesions induced by Virus Diseases (pp. 752–758)



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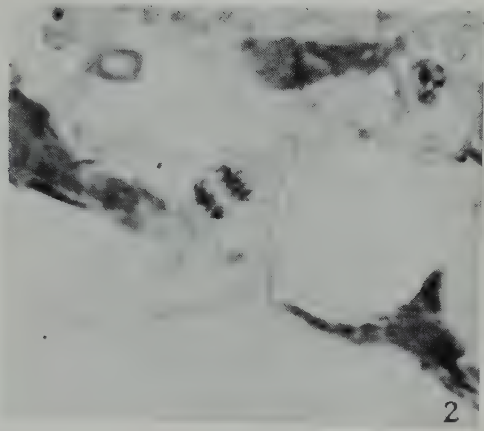

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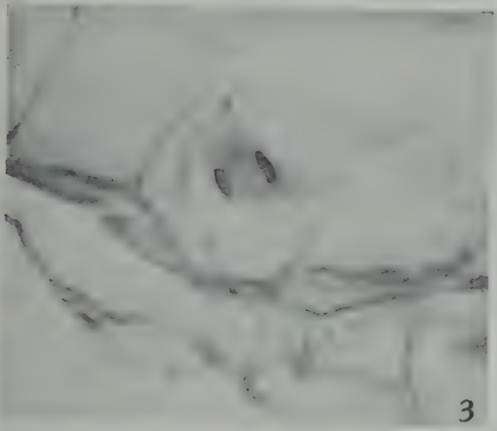

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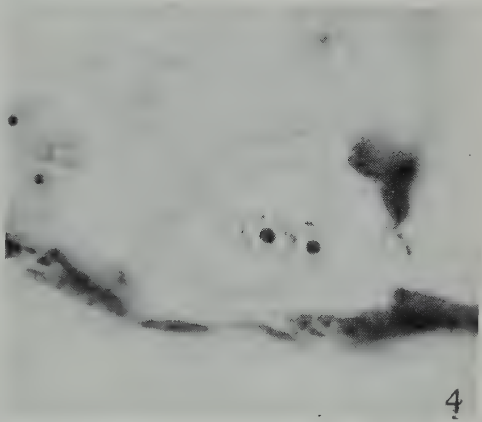

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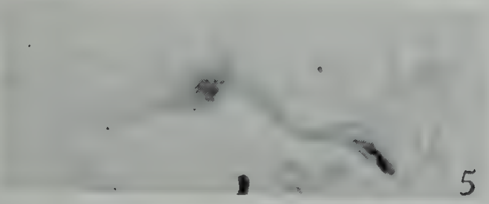

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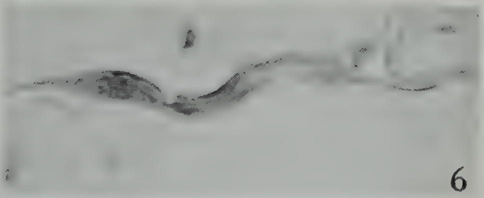

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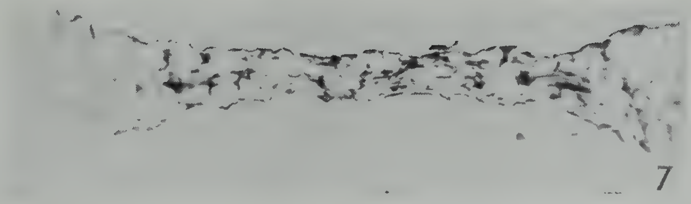

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SHEFFIELD.—The Histology of the Necrotic Lesions induced by Virus Diseases (pp. 752–758)

THE EFFECT OF VARIOUS CHEMICAL TREATMENTS ON THE ACTIVITY OF THE VIRUSES OF TOMATO SPOTTED WILT AND TOBACCO MOSAIC[1]

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(With Plate XXXVI and 2 Text-figures)

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[1] Glasshouse facilities and part of the cost of these investigations were provided by the Council for Scientific and Industrial Research, Australia.

I. INTRODUCTION

THE previously recorded (2, 11) inactivating action of some oxidizing agents and preservative action of some reducing agents on inocula of tomato spotted wilt virus suggested a fuller investigation of the action on the virus of substances having oxidizing and reducing properties. To this end a number of systems was chosen to cover a wide range of oxidation-reduction potentials, and their action on the virus of tomato spotted wilt was investigated. The main questions for which answers were sought were: Do oxidizing and reducing agents as such have any effect on the activity of the virus itself? If so, is there an oxidation-reduction potential range over which the effects operate, or is there a critical potential above which inactivation occurs and below which it does not? Is the effect of atmospheric oxygen a direct one?

The interpretation of the results obtained led to a little further work with other chemicals and with the virus of tobacco mosaic, the results of which are also recorded in the present paper.

II. RÉSUMÉ OF PREVIOUS WORK[1]

Allard (1) tried the effects of a large number of miscellaneous substances on suspensions of ordinary tobacco mosaic virus, and numerous other workers have carried out similar tests on a smaller scale. These tests have shown that the virus of tobacco mosaic is a particularly resistant one, but little more definite can be learned from them.

Johnson (7, 9) has published evidence to show that tobacco mosaic virus may be inactivated in the soil and in aqueous suspensions by means of an oxidation process in which free oxygen is concerned, and he has further (8, 10) used differences in resistance to chemicals as part of a system of classification of plant viruses.

Bald & Samuel (2) found that suspensions of tomato spotted wilt virus were more rapidly inactivated by the bubbling of air through them than by the bubbling of nitrogen (containing about 1 per cent oxygen). They further examined the effect of hydrogen peroxide and chloramine T, and six substances chosen for their possible reducing properties. Hydrogen peroxide and chloramine T caused a rapid inactivation, but since H_2O_2

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[1] Since this paper was written a report of Stanley's work (12) has come to hand in which he described the reaction of tobacco mosaic virus to a large number of chemicals. In view of the absence of statistical tests and the variability of his controls it would be difficult to arrive at definite conclusions in many cases, but where there have been undoubted effects such as for $KMnO_4$ and chlorazene his results are in accord with ours, and the remarks we made concerning our own results may therefore be applied to his also.

is capable of destroying many organic substances and a chloramine T solution gives off free chlorine, no very useful conclusions can be drawn from these results. Of the six "reducing" agents tested, four (including cystein) caused a rapid inactivation of the inocula, one ($NaNO_2$) had a doubtful effect, and one (Na_2SO_3) markedly reduced the normal rate of inactivation. Working under more strictly controlled conditions in buffered suspensions Samuel *et al.* (11) showed that the inactivating effect of some of these reducing agents could be ascribed to the acidity developed by the addition of the test substances. The effect of Na_2SO_3, however, was confirmed and sufficient other evidence obtained to warrant a systematic investigation of the effects of oxidizing and reducing systems in general.

III. EXPERIMENTAL TECHNIQUE

The source of virus, the choice and arrangement of test plants, the general method of preparing inocula and the technique of inoculation were as described in previous papers (3, 11).

Exclusion of oxygen and purification of gases

In the present work the exclusion of traces of oxygen from suspensions of tomato spotted wilt virus was essential. The method used to keep suspensions of this virus in an atmosphere free from oxygen is illustrated in Plate XXXVI. Either hydrogen or nitrogen was used as the inert gas. Commercial nitrogen was drawn from a cylinder of the compressed gas, and was passed first through two bubblers containing 10 per cent $Na_2S_2O_4$ in 10 per cent NaOH solution, and thence through a bubbler of distilled water to a silica tube packed with short lengths of fine copper wire, maintained at 700° C. by means of an electric furnace (calibrated by means of a thermocouple). The gas then passed through another bubbler of oxygen-free distilled water and on into the reaction or storage vessels containing the inocula. Joints in the line of gas flow were reduced to a minimum consistent with flexibility, and were made by winding pure rubber strip,[1] suitably warmed, around the abutting ends of glass or annealed copper tubing and subsequently coating this with colourless duco. The final junction to the storage vessels was made through mercury seals. The rate of bubbling was regulated by carefully ground glass taps. The storage vessels were fitted with carefully ground-in glass stoppers, and aliquots were withdrawn from the vessels through glass taps fitted near the bottom. During the period of storage the purified gas passed continuously through the suspensions, escaping through a water-filled bubbler attached to a side tube of the vessels. When hydrogen was used the purification process was similar, except that the gas was passed directly into the silica tube without first passing the $Na_2S_2O_4$ solution.

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[1] Ordinary india-rubber tubing being quite permeable to oxygen cannot be used. The kind of joint used in this work was suggested to us by the Physics Department of the University of Adelaide and is in common use by physicists for high vacuum work.

The purity of the gases and the efficiency of the oxygen exclusion was tested in various ways. For example, a solution of sodium pyrogallate (*p*H 7), and a solution of K indigo disulphonate in buffer at *p*H 7, reduced by the addition of a solution of $Na_2S_2O_4$ to the stage where a faint green tinge was just noticeable, were enclosed in two of the vessels and hydrogen bubbled through for 20 hours at the rate normally used in these experiments. In neither case was there a change in the depth of the colour at the end of this time, although as soon as the vessels were opened to air by the removal of the stopper both solutions changed colour, that of the indigo disulphonate taking on a deep blue tinge at the surface within a few seconds. Similar results were obtained for the purified nitrogen. Besides being a test of the gases these experiments showed that the whole apparatus was oxygen proof within the limits of the above sensitive tests.

Preparation of inocula

Where the effect of any particular chemical was being investigated solutions were made in a composite buffer (0·04 *M*) at *p*H 7. The buffer solution was pipetted into the reaction vessel through which purified gas was passing, the weighed amount of solid was added and the gas passed for a period of 1 hour or more, and finally the appropriate volume of freshly expressed infective juice was added. During this latter process some oxygen would undoubtedly enter the vessels but would soon be swept out by the gas stream. The time taken from crushing up the diseased leaves until the expressed juice was added to the reaction vessels was never more than a few minutes, and was always reduced to a minimum.

Inocula of tobacco mosaic were prepared in the same way except that in general no attempt was made to exclude air, since it was shown previously(3) that suspensions buffered at *p*H 7 and exposed to air do not change in activity over a period of at least 24 hours.

*p*H *value of the suspensions*

The *p*H values of all suspensions for both viruses was 7. When substances possessing acid or basic properties were to be added to the buffer solution the *p*H value of this was so chosen that after the addition of the substance in question the *p*H value of the resultant solution was 7. Therefore the virus itself was always added to a solution of *p*H 7. The *p*H value of all inocula was checked by means of a spot plate and frequent additional checking by means of the hydrogen or quinhydrone electrodes when possible.

Oxidation-reduction potentials of inocula

On account of the impracticability of determining the oxidation-reduction potentials of the inocula as used, duplicate suspensions were made up which were comparable with those tested in all respects except that they were carried out later and with different samples of juice. In all cases, however, the infective juice was obtained in the same way from the same variety of tomato plant, raised under comparable conditions and at a comparable stage of the disease.

Duplicate electrodes of bright platinum were used, and to make the conditions comparable with the inactivation tests both hydrogen and nitrogen were used as inert gases. In the case of $K_2Cr_2O_7$, for example, hydrogen could not be used on account of its reducing action, setting up a highly negative potential at the electrode even though a large excess of the oxidized form was present. Readings were taken before the addition of infective juice and thereafter at intervals for 4 hours.

The figures given in column 1 of Table II are the oxidation-reduction potentials after 4 hours' contact. A 3·5 *M* solution of KCl was used to minimize liquid junction potentials.

In all cases where the oxidant had a high tinctorial power (as in the case of all those substances used as indicators) the colour of the suspensions at the end of the experiment indicated that the bulk of the test substance was still in the oxidized form.

IV. EXPERIMENTAL RESULTS

A. *Tomato spotted wilt virus*

(1) *The effect of free oxygen on the activity of suspensions of the virus at* *p*H 7.

Suspensions of the virus, even when buffered at *p*H 7, lose their activity rapidly on exposure to air. The time for complete inactivation varied with the initial strength of the inoculum and the temperature.

In one experiment the exposed inoculum was inactive after 6 hours at 15° C., whereas a control inoculum from which oxygen was excluded was still quite active. In another experiment carried out at 22° C. three suspensions, A, B and C, were prepared in buffer solutions of *p*H 7. Suspension A was exposed to air; nitrogen containing approximately 1 per cent oxygen was bubbled through B; and C was maintained in an atmosphere free from oxygen by passing purified nitrogen through it. Suspension A was completely inactive at the 4-hour inoculation. Suspension B was very much more active at corresponding times over the first 4 hours and at the 6-hour inoculation, but was inactive after 12 hours. The activity of suspension C was not significantly different from that of suspension B up to the 6-hour inoculation, but was still active after 12 hours. However, it produced considerably fewer lesions than it did at the first inoculation. At the 24-hour inoculation this suspension produced no lesions.

It is clear that the presence of molecular oxygen hastens the rate of inactivation and that exclusion of oxygen delays it, but there is present in the juice something which causes inactivation even in the absence of oxygen. It was found, however, that when suspensions from which oxygen was excluded were kept at 0° C. by means of a packing of crushed ice they usually maintained their activity unimpaired for much longer periods. The result of an experiment in which air and purified nitrogen were bubbled through two suspensions maintained at 0° C. is recorded in Table I and Text-fig. 1. Before passing through the test inoculum the air was passed first through a bubbler containing 10 per cent NaOH solu-

Table I

A. *Rate of inactivation at* 0° C. *of suspensions of tomato spotted wilt virus (buffered at* *p*H 7) *in the presence and in the absence of oxygen*

Time ...	9 min.		26 min.		1 hr.		1 hr. 37 min.		2 hr.		4 hr.		6 hr.		10 hr. 52 min.	
	N_2	Air	N_2	Air	N_2	Air	N_2	Air	N_2	Air	N_2	Air	N_2	Air	N_2	Air
No. of lesions on16 half-leaf replications	397	352	412	370	403	401	492	432	485	340	473	381	421	249	401	131
% initial value of control (N_2)	100	88·7	104	93	101·5	101	124	109	122	85·6	119	96	106	63	101	33

B. *Summary of analysis of variance*

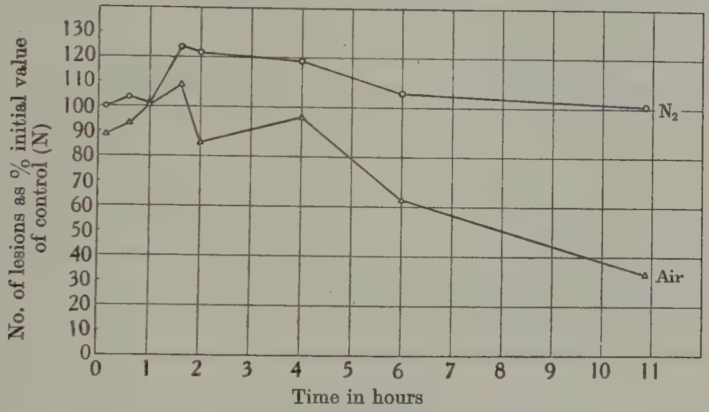
Mean difference (N_2–Air)	2·81	2·63	0·13	3·75	9·06	5·75	10·75	16·88

S.E. of each mean = 2·69. When diff. = 7·40, $P = 0{\cdot}01$ and when diff. = 6·61, $P = 0{\cdot}02$.
S.E. of diff. between means = 3·80. When diff. = 10·46, $P = 0{\cdot}01$ and when diff. = 9·35, $P = 0{\cdot}02$.

The experiment was performed with two 8 × 8 Latin squares on 64 two-leaved (trimmed) tobacco plants using the half-leaf method.

tion and then through a bubbler of distilled water. The nitrogen passing through the control inoculum was purified as previously described.

In plotting the results the number of lesions for each time has been expressed as a percentage of the number of lesions produced by the control inoculum (stored under purified nitrogen) at the first inoculation. Table I B shows that over the first hour and a half there was no significant difference between test and control inocula, but at the 2-hour, 6-hour and 12-hour inoculations the differences were significant, the aerated suspension producing progressively fewer lesions, whereas the control suspension had not changed significantly over the whole period of the experiment. There can be no doubt that the fall in the activity of the aerated suspension was caused by the oxygen, but the form of the inactivation curve suggests that the action is probably not direct. The initial lag could be explained by assuming the initial virus concentration to correspond with a point on the flatter portion of the dilution curve, but since the average number of lesions at the first inoculation was 25 per half-leaf and the test plants were in good condition this is very unlikely. There are not enough points after the fall had set in to determine the form of the inactivation curve, but it does not appear to be logarithmic. The rise in the number of lesions for both inocula during the first 90 min. is not statistically significant in either case, but the frequent occurrence of this phenomenon suggests that it is significant when taken on the whole, and if so is best explained by a mechanical dispersion process following the addition of the juice to the buffer solution. This process may be expected to continue for some time.



Text-fig. 1. Showing changes in the activity of suspensions of tomato spotted wilt virus at *p*H 7 and 0°C. in the presence of (1) nitrogen and (2) air.

(2) *The effect of oxidizing agents in the absence of free oxygen.*

Preliminary experiments (11) had shown that certain oxidizing agents which do not normally destroy organic matter cause inactivation of suspensions of this virus. The present work was designed to determine whether this action was in any way related to the oxidation-reduction potential of the resultant suspension. For this purpose a number of substances was selected to cover a wide range of oxidation-reduction potentials, the majority of these forming reversible systems. As far as was possible this range included substances having the one property of an oxidizing agent in common, but differing in constitution and other properties. At the same time the range included a number of substances of similar constitution but operating over different potential ranges, such as the indophenol series whose oxidation-reduction relationships have been worked out by Clark *et al.* (6). The substances were used in the fully oxidized state, but check tests on some of them in which equimolecular proportions of oxidized and reduced forms were used gave the same results.

In all cases the infective juice was added to solutions of the test substances in composite buffer (pH 7) which had been freed from dissolved oxygen. The suspensions were kept in an atmosphere of purified hydrogen, and aliquots were withdrawn for inoculation soon after preparation and again about 4 hours after the addition of the juice. A control suspension differing only from the test suspensions by the exclusion of the test substance was prepared for each test system, and the two suspensions were inoculated against each other on opposite half leaves of six two-leaved tobacco plants at each inoculation. The results are summarized in Table II A. It will be noticed that the activity of the control suspensions falls with time even in the absence of free oxygen. The fall in activity in test suspensions with a potential greater than +0·2 volt, and also in the case of methylene blue, is, however, much greater, and of a totally different order. The initial fall is greater in some cases than in others, and although there is a general correspondence between the magnitude of the *initial* fall and the oxidation-reduction potential of the suspension, specific effects of the test substances would appear to be equally important.

(3) *The effect of reducing agents in the absence of free oxygen.*

Some substances noted for their reducing powers were tested in the same way as the oxidizing agents except that they were used in their fully reduced form. These systems were chosen to cover a wide potential

Table II

Effect of oxidizing and reducing agents on the activity of suspensions of the virus of tomato spotted wilt in the absence of free oxygen

All suspensions were buffered at *p*H 7. Room temperature.
Numerator =lesions produced by test inoculum (mostly 12 half-leaf replicates).
Denominator = lesions produced by control inoculum (mostly 12 half-leaf replicates).

E_h (volts)	Test substance	Molar conc. of test substance	No. of primary lesions 0	1 hr.	4 hr.
	A. Test substances added in the oxidized form.				
	Iodine + KI	0·001 (I_2)	$\frac{79}{224}$*	—	$\frac{3}{242}$*
+0·49	$K_2Cr_2O_7$	0·005	$\frac{21}{236}$*	$\frac{2}{280}$*	$\frac{1}{43}$*
+0·43	$K_3Fe(CN)_6$	0·005	$\frac{44}{343}$*	—	$\frac{0}{73}$*
+0·32	Quinone	0·005	$\frac{0}{658}$*	$\frac{2}{447}$*	$\frac{1}{154}$*
+0·25	*o*-Bromophenol indophenol	0·001	$\frac{33}{580}$*	$\frac{5}{634}$*	$\frac{0}{199}$*
+0·25	Phenolindophenol	0·001	$\frac{286}{487}$*	—	$\frac{0}{391}$*
+0·25	*o*-Cresolindo-2-6-dichlorophenol	0·001	$\frac{67}{574}$*	—	$\frac{2}{177}$*
+0·23	*l*-Naphthol-2-sulphonate indophenol	0·001	$\frac{62}{140}$†	—	$\frac{20}{61}$
+0·19	*l*-Naphthol-2-sulpho-indo-2-6-dibromophenol	0·001	$\frac{754}{819}$	—	$\frac{101}{291}$*
+0·12	Thionine	0·001	$\frac{523}{505}$	—	$\frac{264}{181}$
+0·044	Methylene blue	0·001	$\frac{186}{858}$*	—	$\frac{4}{644}$*
−0·09	K indigo disulphonate	0·001	$\frac{777}{874}$	—	$\frac{439}{394}$
−0·21	Neutral red	0·001	$\frac{57}{135}$†	—	$\frac{40}{61}$
	B. Test substances added in the reduced state.				
+0·12	Na pyrogallate	0·01	$\frac{116}{100}$	$\frac{92}{72}$	—
+0·06	Na_2SO_3	0·0025	$\frac{723}{658}$	$\frac{427}{447}$	$\frac{442}{154}$*
−0·17	Cystein	0·01	$\frac{271}{187}$	—	$\frac{270}{31}$*
−0·41	H_2—Platinized Pt	—	$\frac{65}{32}$*	—	$\frac{70}{18}$*
−0·42	$Na_2S_2O_4$	0·01	$\frac{528}{664}$	—	$\frac{346}{444}$
			0	1½ hr.	6 hr.
	$Na_2S(NaHS)$	0·01	$\frac{252}{312}$	$\frac{234}{171}$	$\frac{184}{78}$†

* P = or $<0·01$. † P = or $<0·02$.

range, and substances known to have effects on plant juices other than those being investigated were avoided (e.g. tannic acid, because of its action as a protein precipitant). The number of substances available was limited, but the results set out in Table II B show that from Na-pyrogallate and Na_2SO_3 to the hydrogen-platinized platinum system and $Na_2S_2O_4$ the effect was essentially the same. Not only was there no increase in the inactivation rate in these systems but in some cases the activity of the test suspensions containing these substances fell off less rapidly than did that of the controls.

Considering Tables II A and II B together it will be seen that of the substances added in the oxidized state all of those which gave a suspension with an E_h value above $+0{\cdot}2$ volt caused a rapid inactivation of the virus, whereas those with E_h values near to and below $+0{\cdot}1$ volt (methylene blue excepted) either failed to inactivate the virus at all (as compared with the controls) or did so to a very much less extent than those with higher E_h values.

The case of neutral red is somewhat puzzling, since there was a significant effect during the first 10 min., but at the 4-hour inoculation there was no significant difference between test and control inocula. Between the "zero time" and 4-hour inoculations the proportionate fall in activity of the test inoculum was in fact less than that of the control. The initial effect therefore appears to be due in this case either to an effect of the reagent on the test plant or to some impurity present in small quantity. It must be remembered that the first reading of a potential is taken about 1 min. after the addition of the juice; any small quantity of impurity possessing a higher oxidation-reduction potential would probably be reduced by this time and yet the amount may have been sufficient to inactivate the virus.

If, for the present, we exclude methylene blue from the discussion, the order of the induced potentials is seen to correspond with the inactivating effect on the virus.

With regard to methylene blue it should be noted that this thiazine differs from thionine constitutionally only by the substitution of four methyl groups for hydrogen atoms. Thionine operates at a more oxidizing potential and yet has no inactivating effect whatever on the virus. Clark *et al.* (5) have shown that methylene blue is sensitive to light and undergoes photochemical oxidation. In the presence of a substance which will reduce methylene blue we may therefore get a reduction of the dye followed by an oxidation under the influence of light to a reactive oxidized form. Experiments in which light was excluded up to the moment of inoculation have shown that the results obtained above are not likely to have been due to this effect, since inactivation takes place even under these conditions.

The behaviour of those substances which were added in the reduced form fits in with the idea that inactivation of the virus takes place only at relatively high oxidation-reduction potential values. The substances listed in Table II B not only fail to inactivate the virus but they actually arrest the normal anaerobic inactivation as observed in the control suspensions, at least over the relatively short time covered by these experiments. It appears from this that the cause of the falling off in the activity of the control suspensions *in the absence of free oxygen* is due to the presence in the juice of some oxidized substance (or substances), that this substance is usually present in only small, but variable, amount, and that it is reduced by those substances listed in Table II B, thus preventing further inactivation.

(4) *The effect of reducing agents in the presence of air.*

However, the effect of reducing systems in the presence of air was of greater interest in this study. It will be seen from Table III that the presence of these reducing agents markedly retarded the rate of inactivation of suspensions of the virus. This effect was common to all systems, although some appeared to be more efficient as protective agents than others.

Table III

Effect of reducing systems on the activity of suspensions of the virus of tomato spotted wilt exposed to air

All suspensions were buffered at *p*H 7. Concentration of test substances = 0·01 *M*.
Numerator = lesions produced by test inoculum (8 half-leaf replicates).
Denominator = lesions produced by control inoculum (8 half-leaf replicates).

	No. of hours				
Test substance	0	1½	3	6	12
Na_2SO_3	$\frac{432}{369}$	$\frac{357_*}{101}$	$\frac{264_*}{11}$	$\frac{196_*}{4}$	—
Na pyrogallate	$\frac{311}{440}$	$\frac{199}{127}$	$\frac{64_*}{5}$	$\frac{8}{0}$	—
Cystein	$\frac{313_*}{264}$	—	$\frac{317_*}{208}$	$\frac{443_*}{6}$	$\frac{359_*}{0}$
$Na_2S_2O_4$	$\frac{303_*}{189}$	$\frac{306_*}{66}$	$\frac{92_*}{3}$	$\frac{105_*}{1}$	—
$Na_2S(NaHS)$	$\frac{71}{75}$	$\frac{28}{26}$	$\frac{25}{5}$	$\frac{2}{0}$	—

* $P =$ or $< 0{\cdot}01$.

Cystein, Na_2SO_3, $Na_2S_2O_4$ and Na_2S are all sulphur compounds. It so happens that most of the easily accessible reducing agents operating over this potential range, which are at the same time not open to objection on account of some other possible action, are sulphur compounds, but since the pyrogallate and the hydrogen-platinum systems also protect the virus (the latter in the absence of oxygen, but Table II B shows the protective effect as compared with the control also in the absence of oxygen) the effect may be ascribed to their common action as reducing agents. There is, however, an important difference between pyrogallate and these sulphur compounds. On exposure to air they all oxidize, but the oxidation products of Na_2SO_3, cystein, $Na_2S_2O_4$ and Na_2S are not active oxidants. The oxidation products of pyrogallate are complex but contain quinones as intermediate products and these may be expected to inactivate the virus, thus setting up opposing tendencies. With Na_2S and $Na_2S_2O_4$ the *p*H values of inocula are more difficult to control, and in any case some free H_2S is produced after some time and the *p*H value of the suspensions tends towards lower values. Secondary changes taking place in these systems may therefore be the reason why these two substances are not such good protective agents as Na_2SO_3 or cystein.

In two separate experiments on the preservative action of cystein, inoculations performed 10 and 48 hours respectively after adding the virus to the test solutions resulted in significant increases in the number of lesions above the number for the 10-min. inoculations. The increased number of lesions may, however, be accounted for by differences in the environmental conditions to which the test plants were exposed before and after inoculation (4, 11). The possibility that some part of the increases may have been due to multiplication of the virus *in vitro*, or to a reactivation of virus inactivated in the plant before the juice was expressed, or in the juice during transfer to the test solution, seems therefore improbable, but cannot be ruled out entirely.

(5) *Miscellaneous tests.*

In order to obtain some information on the mode of inactivation of control suspensions when exposed to air, tests were carried out with substances such as NaF and KCN which are known to inhibit certain respiratory enzymes. In addition the effect of the well-known poison, $HgCl_2$, was tested. Mercury was precipitated as HgS before inoculation of such suspensions, and it therefore became necessary to test the effect of H_2S alone. The preservative action of KCN was open to several interpretations, making further tests necessary.

Catechol was used in the first instance as a reducing agent operating at a potential more positive than the other reducing agents, but it soon became evident that the action of this and similar substances was complicated by the production of oxidation products, and tests were carried

Table IV

Miscellaneous tests. Effect of various chemical treatments on the activity of suspensions of tomato spotted wilt virus

All suspensions were buffered at *p*H 7 and (except where otherwise indicated) were exposed to air. Where only one substance appears in column 1 this means that the control suspension was simply made up in buffer solution of *p*H 7.

Test system	No. of half-leaf replicates	$\frac{\text{Lesions from test suspension}}{\text{Lesions from control suspension}}$ Hours					
		0	$1\frac{1}{2}$	3	4	6	12
0·01 *M* $NaNO_2$	8	$\frac{122}{159}$	$\frac{97}{51}$	$\frac{22}{21}$	—	$\frac{7}{1}$	—
0·01 *M* NaF	8	$\frac{129}{134}$	—	$\frac{49}{46}$	—	$\frac{0}{0}$	$\frac{0}{0}$
Saturated H_2S	12	$\frac{159}{172}$	—	—	$\frac{11}{122}$ *	—	—
0·001 *M* $HgCl_2$ (absence of oxygen)	12	$\frac{0}{123}$ *	—	—	$\frac{0}{54}$ *	—	—
0·01 *M* KCN (absence of oxygen)	12	$\frac{130}{77}$ *	—	—	$\frac{155}{14}$ *	—	—
0·01 *M* KCN (air)	8	$\frac{380}{223}$	—	$\frac{371}{174}$ †	—	$\frac{157}{4}$ *	$\frac{76}{0}$ *
Saturated cystine	16	$\frac{952}{1102}$	$\frac{856}{830}$	$\frac{279}{280}$	—	$\frac{23}{13}$	—
$\frac{0{\cdot}01\,M\ \text{KCN} + \text{cystine (saturated)}}{0{\cdot}01\,M\ \text{KCN}}$	8	$\frac{121}{124}$	$\frac{112}{55}$ *	$\frac{39}{5}$	—	$\frac{37}{0}$ †	—
0·01 *M* catechol	8	$\frac{2}{473}$ *	$\frac{2}{70}$ *	$\frac{0}{11}$	—	$\frac{0}{0}$	—
0·01 *M* catechol + 0·01 *M* Na_2SO_3	—	$\frac{318}{366}$	—	$\frac{303}{152}$ *	—	$\frac{329}{0}$ *	$\frac{344}{0}$ *
0·01 *M* catechol + $\frac{0{\cdot}01\,M\ Na_2SO_3}{0{\cdot}01\,M\ Na_2SO_3}$	8	$\frac{640}{543}$	$\frac{316}{318}$	$\frac{439}{256}$	—	$\frac{355}{385}$	—
0·01 *M* catechol + $\frac{0{\cdot}01\,M\ \text{KCN}}{0{\cdot}01\,M\ \text{catechol}}$	8	$\frac{97}{0}$ *	$\frac{19}{0}$ †	$\frac{0}{0}$	—	$\frac{0}{0}$	—
0·01 *M* phenol	12	$\frac{0}{334}$ *	—	—	$\frac{0}{6}$	—	—
0·01 *M* phenol + $\frac{0{\cdot}01\,M\ Na_2SO_3}{0{\cdot}01\,M\ Na_2SO_3}$	12	$\frac{347}{389}$	—	—	$\frac{142}{165}$	—	—
0·01 *M* KCN + $\frac{0{\cdot}001\,M\ HgCl_2}{0{\cdot}01\,M\ \text{KCN}}$	8	$\frac{42}{137}$ *	$\frac{8}{79}$ *	—	—	—	—

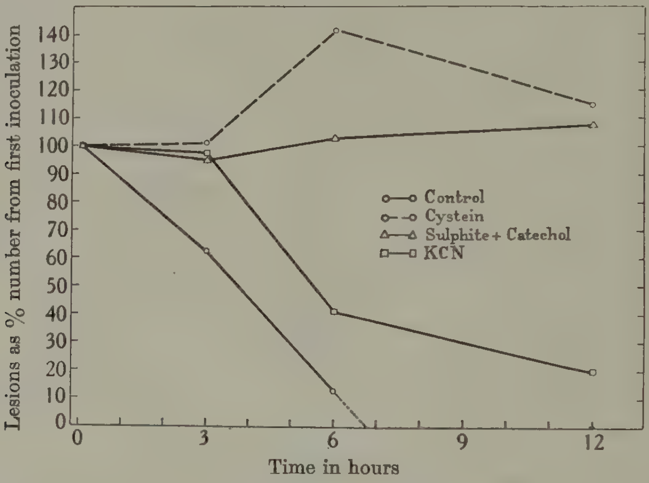
Notes. H_2S: a continuous stream of gas was passed through the inoculum.
Cystine: saturated solutions were used + a slight excess of solid.

* *P* = or < 0·01. † *P* = or < 0·02.

out to elucidate their behaviour. They have therefore been included in this section rather than in that dealing with simple reducing systems. The results of all these tests are grouped together in Table IV.

$NaNO_2$. Under controlled conditions $NaNO_2$ has no effect on virus activity. The slight preservative effect reported by Bald & Samuel (2) must therefore have been due to other effects, possibly to differences in *p*H value between test and control inocula.

NaF. At 0·01 *M* concentration and at *p*H 7 NaF had no effect on virus activity.



Text-fig. 2. Activity-time curves for suspensions of tomato spotted wilt virus at *p*H 7 in the presence of various chemicals (0·01 *M*). All inocula exposed to air at room temperature.

H_2S. The inactivating effect of this substance may be partly or wholly due to the acidification which results when H_2S is passed through the inoculum (even when buffered). The point of interest for the present is that the action on the virus is a fairly slow one.

$HgCl_2$. Mercuric chloride at a concentration of 0·001 *M* causes *instantaneous* inactivation, and the inactivated virus was not reactivated by precipitating the Hg with H_2S.

KCN. On the other hand, KCN has a marked protective effect. Besides arresting the normal slow, anaerobic inactivation it actually protects the virus against inactivation on exposure to air. Its effect is, however, not so prolonged as is the case with Na_2SO_3 or cystein. Text-fig. 2 brings out this difference clearly.

If the respiratory enzymes present in the inoculum were concerned in the aerobic inactivation and the effect of KCN was indirect through its action on these, we should expect the protective effect to be more lasting. We must therefore look elsewhere for the explanation.

One well-known property of KCN is to enter into reaction with organic disulphides to form sulphydril compounds. Since the unpurified juice has been used for the preparation of inocula it is possible that KCN may react with cystine and other similar compounds, thus indirectly producing reducing agents of the type which we have shown protect the virus. To test this point several experiments were carried out in which infective juice was added to solutions of buffer at pH 7, buffer + solid cystine, buffer + cystine + KCN and buffer + KCN. The results (Table IV) show that the presence of cystine alone is without effect, whereas when KCN is added as well the suspension has a protective effect which is greater (extending over a longer period) than when KCN alone is added. It therefore seems highly probable that the action of KCN is along the lines suggested. The reason for the relatively short period over which it exerts a protective action is to be found in the relatively small quantities of sulphydril compounds released from constituents of the juice.

Catechol and phenol. The action of these substances may conveniently be considered together. They cause a rapid inactivation of the virus when exposed to air. In the absence of oxygen the effect is similar but not quite so pronounced.

In the presence of Na_2SO_3 their inactivating effect is inhibited. Reference to Table IV and Text-fig. 2 shows, for example, that a suspension of the virus in catechol ($0{\cdot}01\,M$) + Na_2SO_3 ($0{\cdot}01\,M$) in buffer at pH 7 was just as active after 12 hours' exposure to air as it was 10 min. after preparation. A similar effect was obtained with an $0{\cdot}0025\,M$ solution of Na_2SO_3 in presence of $0{\cdot}01\,M$ quinol.

Suspensions of infective juice in phenol, catechol and quinol solutions (pH 7) all develop a dark colour on exposure to air. They do not do so in the absence of oxygen or in the presence of oxygen when Na_2SO_3 is also present in sufficient concentration. Solutions of quinol and catechol alone in buffers of pH 7 are known to undergo autoxidation. The rate of production of coloured oxidation products is also much greater in the presence of the juice than in its absence. It appears therefore that there is present in the juice added along with the virus some substance (or group of substances) of the nature of a catalyst (or enzyme) which hastens the oxidation of these compounds, thus producing quinones or similar substances, which then rapidly inactivate the virus.

Effect of temperature. The fact that the rate of inactivation of suspensions in the absence of oxygen varied from experiment to experiment presented a difficulty which required investigation. To this end the effect of temperature on the rate of inactivation in the absence of oxygen was investigated.

The rate of inactivation was found to be greater at the higher temperatures. The difference would not account for the great range of variation encountered, but showed that temperature differences were a contributing factor.

(6) *Attempts to reactivate virus which has been inactivated by various means.*

Suspensions of the virus were exposed to air and at various stages of the inactivation process these were subdivided and attempts made to reactivate the inactivated virus by adding Na_2SO_3, cystein or $Na_2S_2O_4$. In case any possible reactivation process had a lag period inoculations were made in some instances at suitable intervals after the addition of the solutions under test. In no case was there any significant difference between test and control suspensions. It is clear therefore that virus inactivated by exposure to air cannot be reactivated by the addition of such reducing agents as we have used. Reference to Table II B shows that these three substances operate at widely different potentials and cover the range down to the potential of the hydrogen electrode. It is therefore very unlikely that the virus can be reactivated by these means.

As pointed out previously, virus which has been inactivated by $HgCl_2$ cannot be reactivated by precipitation of the Hg by H_2S.

(7) *Tests to determine whether the effects observed are on the virus itself.*

On account of the rapid loss in infectivity of the juice expressed from diseased tomato plants, inocula were prepared by adding the juice direct (after filtration through muslin) to the suspension media. It is therefore possible that the inactivating action of some chemicals may have been due to an action on some constituent of the juice other than the virus, such as precipitation of an ingredient which then carried the virus down with it. In order to test this possibility the following series of experiments was carried out.

Suspensions of tomato spotted wilt virus were prepared in buffer solution alone, buffer solution + $K_2Cr_2O_7$ (to make an $0{\cdot}0005\,M$ solution), buffer solution + $K_3Fe(CN)_6$ ($0{\cdot}005\,M$) and buffer solution + cystein ($0{\cdot}01\,M$). Soon after preparation and again after 4 hours' contact these suspensions were inoculated to *Nicotiana tabacum* and *N. glutinosa* plants. Oxygen was excluded from these suspensions. At the same time suspensions of tobacco mosaic virus prepared from the untreated juice of diseased tobacco plants were made up in the same media to form a second series of inocula, and suspensions of tobacco mosaic virus similarly prepared from the untreated juice of tomato plants (same variety as used for tomato spotted wilt) artificially infected with tobacco mosaic formed a third series of inocula. Suspensions from the second and third series were inoculated on to *N. glutinosa* plants soon after preparation and again after 4 hours' contact. The results are set out in Table V.

Table V

Showing that the action of certain chemicals is on the virus of tomato spotted wilt itself

Numerator =No. of lesions produced by test inoculum.
Denominator=No. of lesions produced by control inoculum.

Test substance (buffered at pH 7)	Tomato spotted wilt on *N. tabacum*		Tomato spotted wilt on *N. glutinosa*		Mosaic from tomato plants		Mosaic from tobacco plants	
	Time of contact (hours)							
	0	4	0	4	0	4	0	4
0·005 M $K_2Cr_2O_7$	$\frac{40}{378}$ *	$\frac{1}{220}$ *	$\frac{29}{255}$ *	$\frac{5}{96}$ *	$\frac{854}{1046}$	$\frac{1173}{1631}$ *	$\frac{851}{1040}$ †	$\frac{664}{997}$ *
0·005 M $K_3Fe(CN)_6$	$\frac{44}{343}$ *	$\frac{0}{73}$ *	$\frac{44}{214}$ *	$\frac{4}{56}$ †	$\frac{1424}{1372}$	$\frac{1083}{1110}$	$\frac{673}{869}$	$\frac{1085}{1315}$
0·01 M cystein	$\frac{757}{335}$	$\frac{515}{113}$ *	$\frac{312}{227}$	$\frac{258}{45}$ *	$\frac{979}{1094}$	$\frac{958}{824}$	—	—

* P = or $<0{\cdot}01$. † P = or $<0{\cdot}02$.

Inoculation of suspensions of tomato spotted wilt containing the test substances on to *N. glutinosa* plants gave the same results as when inoculated on to tobacco plants. This, together with the fact that a number of lesions are produced at zero time, and the results with mosaic suspensions containing the oxidizing agents, rules out the possibility of any large effect of the chemicals on the host plant as distinct from action on the inoculum. There is also no appreciable difference between the behaviour of mosaic suspensions prepared from tobacco plant juice and from tomato plant juice.

In the tests with tobacco and "tomato" mosaic virus, $K_3Fe(CN)_6$ gave results which are not significantly different from the controls. The same applied to cystein with "tomato" mosaic. The presence of $K_2Cr_2O_7$, however, caused a small but significant reduction in the number of lesions produced by both "tomato" and tobacco mosaic suspensions after 4 hours' contact. The difference between test and control at the 4-hour inoculation is not significantly different from the difference between test and control at the first inoculation. The major effect would therefore appear to be one on the host plant. Nevertheless the fact that the individual differences are only doubtfully, if at all, significant at the first inoculation and clearly so at the second leads one to ascribe something to an effect on the virus itself. Larger effects may therefore be expected with longer times of contact, but the important point for our present purpose is that the total effect is small and the reaction (if any) is slow and in no way comparable with the effect of tomato spotted wilt inocula.

In many cases (Table II A) the number of lesions at the first inoculation is fairly high, and the subsequent fall is clearly due to an action on the virus as distinct from an action on the test plant.

Taken as a whole the results show, as well as it is possible to do so without actually isolating the tomato spotted wilt virus, that the effects observed are, in the main at least, due to an effect on the virus itself.

B. *Tobacco mosaic virus*

The effect of various chemical treatments on the activity of the virus.

Tests similar to those described for tomato spotted wilt virus were carried out on suspensions of ordinary tobacco mosaic virus. The technique was the same except that no attempt was made to exclude air for reasons already mentioned. The purpose of the tests was to see whether there was any reaction of reasonable speed such as would be of use in arriving at information concerning possible reactive groups and the like. Slow actions spread over some days are of little use in this connexion and therefore many of the tests were of short duration only. The results are set out in Table VI.

With the exception of $KMnO_4$ and chlorazene (which amounts to a test of the action of free chlorine) it is clear that oxidizing and reducing agents have no marked effect on this virus. $KMnO_4$ is capable of destroying many organic substances, so that its action on the virus does not give any very useful information.

The slight action of $K_2Cr_2O_7$, discussed in a previous section of this paper, is also of very little use as a means of determining possible chemical affinities of the virus, but all these slow actions taken together and extended may at some future date be of value and so are recorded here. Quinone in hundredth molar solution caused a significant fall in the number of lesions, but in thousandth molar solution it had no effect. Also there is no significant change in the ratio $\frac{\text{lesions from test}}{\text{lesions from control}}$ from the initial to the 6-hour inoculation. The same applies to the action of catechol, although in this case there is no significant effect at any one time. For these two substances it appears that the effects observed are on the host plant and not on the virus before inoculation. Bubbling H_2S through a suspension of the virus for 1 hour had no effect on the number of lesions produced.

Table VI

Effect of various chemicals on the activity of suspensions of ordinary tobacco mosaic virus

All suspensions were buffered at pH 7. Control suspensions made up in buffer solution of pH 7.

Test system	Molar conc. of test substance	$\frac{\text{Lesions from test}}{\text{Lesions from control}}$ Hours				
		0	1	3	4	6
$KMnO_4$	0·01	$\frac{56}{299}$*	$\frac{4}{341}$*	$\frac{2}{251}$*	—	$\frac{0}{326}$*
$KMnO_4$	0·001	$\frac{100}{171}$*	$\frac{89}{199}$*	$\frac{22}{173}$*	—	$\frac{3}{188}$*
$I+KI$	$I=0{\cdot}001\,M$	0·62	0·59	0·65	—	0·88
Chlorazene	0·001	0·42†	0·41*	0·28†	—	0·07*
$K_2Cr_2O_7$ (tobacco mosaic)	0·005	0·82†	—	—	0·67*	—
$K_2Cr_2O_7$ ("tomato" mosaic)	0·005	0·82	—	—	0·72*	—
$K_3Fe(CN)_6$ (tobacco mosaic)	0·005	0·75	—	—	0·83	—
$K_3Fe(CN)_6$ ("tomato" mosaic)	0·005	1·04	—	—	0·98	—
Iodoxybenzene	Saturated	0·95	—	0·81	—	(1·42 after 26 hr.)
Quinone	0·01	0·25*	0·35*	0·24*	—	0·33*
Quinone	0·001	1·14	1·23	0·80	—	0·80
Quinone for 4 hr. then $+Na_2SO_3$	Q=0·001 sulphite =0·01	—	—	—	1·01	—
Catechol	0·01	0·78	0·79	0·78	—	0·65
Na pyrogallate	0·01	—	1·00	—	—	—
Na_2SO_3	0·01	—	1·17	—	—	—
Cystein ("tomato" mosaic)	0·01	0·90	—	—	1·17	—
$Na_2S_2O_4$	0·01	—	0·88	—	—	—
H_2S	Saturated	—	1·03	—	—	—
KCN	0·01	0·89	0·78	0·81	—	0·96
$HgCl_2$	0·001	0·32*	0·32*	0·25*	—	0·34*
$HgCl_2+H_2S$	—	—	1·13	—	—	—

* $P=$ or $<0{\cdot}01$. † $P=$ or $<0{\cdot}02$.

The effect of iodine (in the presence of KI) is rather interesting. After 10 min., 1 hour and 3 hours contact the effect was a reduction in the number of lesions to about 60 per cent of the control inoculum. The fact that the ratio is constant for these three inocula suggests again that the effect is on the host plant, and the fact that at the final inoculation after 6 hours' contact the apparent reduction is only 12 per cent tends to confirm this, since by this time much of the iodine had volatilized (the flasks were exposed to air with only a cotton-wool plug to prevent contamination). Potassium cyanide in 0·01 *M* solution had no significant effect after 6 hours' contact. Mercuric chloride in 0·001 *M* concentration caused a reduction in the number of lesions to 32, 31·5, 25 and 23 per cent of the control after 10 min., 1 hour, 3 hours and 6 hours contact respectively. The *immediate* fall to a constant level again makes one suspect an effect on the host or test plant, and this view was supported by the following experiment. After a 1-hour contact the test suspension was divided into two parts, one was inoculated against the control without further treatment, while the other one was treated with H_2S to precipitate the Hg and was then inoculated (after filtering off HgS) against the control. In all tests the half-leaf method of comparison was used.

The inoculum containing $HgCl_2$ gave about half as many lesions as the control, whereas the inoculum from which the Hg had been precipitated actually gave more lesions than the control, but this difference was not significant. There is still the possibility that the $HgCl_2$ had combined with the virus rendering it temporarily inactive, and that the removal of Hg by H_2S again set free the virus, but this seems a less probable explanation. Contact with $HgCl_2$ for longer periods (up to 2 months) resulted in complete inactivation of the virus. This result is interesting but has no bearing on the short period tests just discussed. Pyrogallate, Na_2SO_3, cystein and $Na_2S_2O_4$ had no effect on virus activity over the short times of contact tested in this work.

V. SUMMARY

A. *Tomato spotted wilt virus*

(*All tests made with suspensions buffered at* pH 7)

1. The concentration of active virus units in suspensions of tomato spotted wilt virus stored at 0° C. in a buffer solution of *p*H 7 was maintained without loss for 11 hours by excluding free oxygen.

2. A significant fall in the concentration of active virus units occurred in suspensions treated as in (1) but through which air was bubbled.

3. At room temperatures the virus underwent fairly rapid inactivation even in the absence of free oxygen, although the presence of oxygen hastened the inactivation. The rate of inactivation in the absence of oxygen varied from time to time, and it is concluded that this inactivation was due to the presence in the infective juice of some oxidized

substance which is usually present in only small but variable amounts, and that it is reduced by those substances (see (4) below) which arrest anaerobic inactivation.

4. Certain substances added in the reduced form which yield suspensions with an E_h value of $+0{\cdot}1$ volt or less at pH 7 arrested the normal anerobic inactivation of the virus. The remarkable preservative action of cystein is discussed.

5. The virus was rapidly inactivated *in vitro* by $0{\cdot}001\,M$ solutions of oxidizing agents which induced in the suspensions a potential greater than $+\,0{\cdot}2$ volt at pH 7. With the exception of methylene blue, oxidizing agents which gave a suspension with an E_h value below $+0{\cdot}1$ volt at pH 7 did not inactivate the virus.

6. The reducing agents referred to in (4) protected the virus against inactivation when exposed to air. This protective effect was not permanent but prolonged the activity of inocula for many hours beyond that of the controls.

7. The effect on the virus of a number of other substances was also examined. Those having greatest interest are: (*a*) KCN, which in $0{\cdot}01\,M$ solution protected the virus both against anaerobic and aerobic inactivation. Its probable mode of action is discussed on the bases of experiments reported. (*b*) $HgCl_2$, which in $0{\cdot}001\,M$ solution caused instantaneous inactivation of the virus. (*c*) Cathecol, quinol and phenol alone inactivated the virus in the presence of air, but did not do so if Na_2SO_3 was also present. It is concluded that secondary oxidation products caused the inactivation observed.

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Sul[illegible]

8. Attempts to reactivate virus which had been inactivated by exposure to air or by means of $HgCl_2$ were unsuccessful.

9. It has been shown that the inactivating effects observed are due to an action on the virus itself.

B. *Tobacco mosaic virus*

(*All tests made with suspensions buffered at* pH 7)

The effect of various chemicals on the activity of tobacco virus 1 was tested, chiefly as a means of comparing it with the virus of tomato spotted wilt.

Of the fifteen chemicals tested only $KMnO_4$ and chlorazene caused a rapid inactivation. Benzoquinone, iodine, $K_3Fe(CN)_6$, iodoxybenzene, KCN ($0{\cdot}01\,M$), and a number of well-known reducing agents covering the potential range down to that of the hydrogen electrode (pH 7), did not affect virus activity over the relatively short time periods tested.

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$K_2Cr_2O_7$ (0·005 M) appeared to have a slight inactivating effect after 4 hours' contact. Contact with $HgCl_2$ (0·001 M) for a few hours did not affect the virus but contact for longer periods caused complete inactivation.

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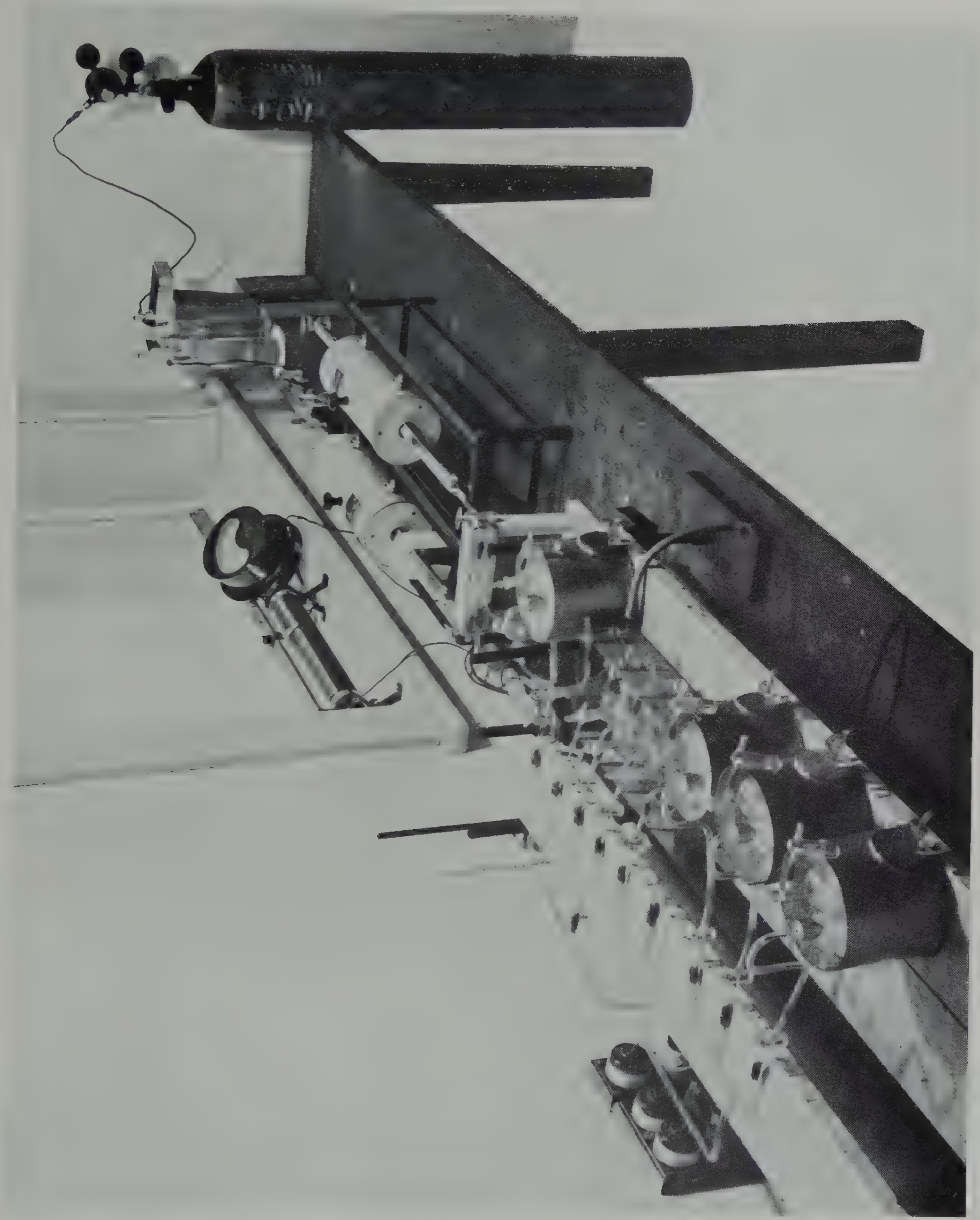
EXPLANATION OF PLATE XXXVI

Showing the gas purifying apparatus, and two types of storage vessel packed in ice.

(*Received* 19 *May* 1936)



BEST AND SAMUEL.—The Effect of various Chemical Treatments on the Activity of the Viruses of Tomato Spotted Wilt and Tobacco Mosaic (pp. 759–780)

ON THE BIOLOGY OF CERTAIN SPECIES OF *HOLCOCNEME*[1] KON. (HYMENOPTERA-SYMPHYTA)

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(With Plates XXXVII and XXXVIII and 4 Text-figures)

Four members of the genus *Holcocneme* Kon. (*Nematus* Panz.) (8) are recorded as British and they may be identified with the aid of the following key:

1. Tarsal claws bifid ....**2.**
   Tarsal claws with a subapical tooth and abdomen with some red dorsally ....
   H. erichsoni Htg.
2. Head with transverse frontal ridge unbroken; pronotum and abdomen black...**3.**
   Head with transverse frontal ridge broadly broken through; pronotum red and abdomen girdled with red...**H. lucida** Panz.
3. The long spur on hind tibia at least half as long as the metatarsus ....
   H. crassa Fall.
   The long spur on the hind tibia at most only one-third the length of the metatarsus
   H. caeruleocarpa Htg.

Holcocneme erichsoni, well known (9) as the large larch saw-fly, occurs during May, June and July and may be sufficiently numerous to cause extensive defoliation in larch plantations. *Holcocneme lucida* occurs in early spring about the time *Prunus* sp. are in flower and appears to be widely distributed but not common. *Holcocneme caeruleocarpa* is common on *Salix* and *Populus* and may be taken from May to August. *Holcocneme crassa* has not been taken by the writer in the north of England, but has been taken occasionally by Perkins (19) on *Salix* in Devonshire. The adults and larvae of *Holcocneme crassa* described in this paper were reared by Mr R. B. Benson, M.A. from *Rumex* in Hertfordshire.

BIONOMICS OF *HOLCOCNEME CAERULEOCARPA* HTG.

Females of this species may be taken on willows and poplars. They fly fairly readily, and generally alight on the leaves and immediately walk over the edge to the under side. Later they rest motionless for long periods on the main stems or on the under surface of the leaves.

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[1] R. B. Benson (1935), *Ent. Mo. Mag.* LXXI, 240 proposes certain alterations in the Nematinae including "*Nematus* Panzer with *N. lucidus* Panzer as type = ...*Holcocneme* Konow...."

Oviposition and incubation

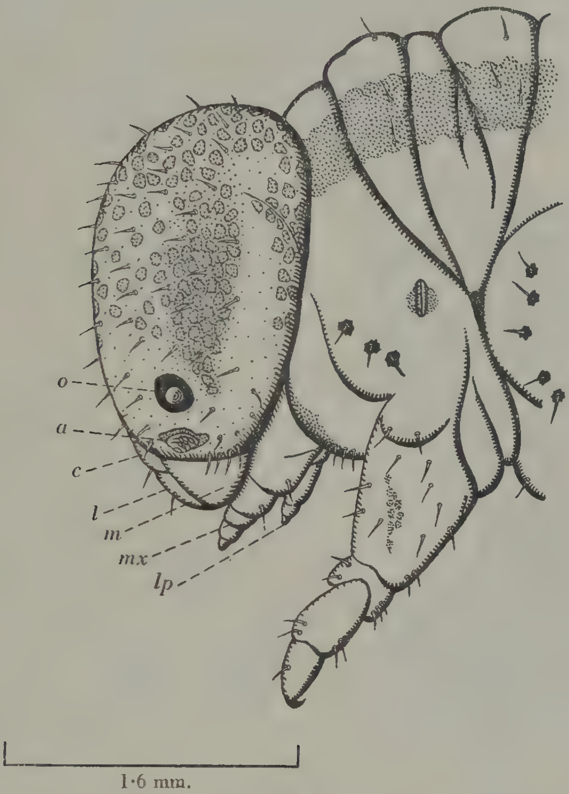
Salix purpurea and *S. fragilis* are most frequently selected by ovipositing females but eggs are also laid upon *S. triandra*, *S. viminalis* and various species of *Populus*. After examining the lower surface of the leaf with the tip of the abdomen, the insect forces the terebra into the tissue, curves the saws forward beneath the epidermis and cuts a pocket-shaped incision. The egg is placed in this prepared cavity.

Eggs are usually laid on the lower surface of the leaves, most frequently between lateral veins and nearer to the mid-rib than the margin, but occasionally just within the margin. At first the egg incisions are not easily seen, but after 2 or 3 days the eggs increase in size and in 5 or 6 days after oviposition the egg site is conspicuous (Pl. XXXVII, fig. 1). The edges of the incision are forced apart during incubation and the outline of the egg becomes visible. The period of incubation for eggs laid during May is 8–9 days.

The larval stages

At eclosion the larva is smoky grey with the head darker than the body, but after 3 or 4 days it appears to be dark green because the green contents of the alimentary canal are visible through the grey integument. The head is black and the legs are dark with dark brown or black tarsal claws. The larva is slender and tapers toward the tip of the abdomen where there is a pair of small, dark green pseudocerci. As in other nematine saw-flies, prolegs occur on segments 5–10 and 13. There are a few short setae on the epipleurites of the body segments and on the vertex of the head. The duration of the first stadium is 3–4 days, and at its close the larva is 5–6 mm. long. When beginning to feed the first-instar larva bites into the leaf tissue from the under side and makes a hole. The hole is gradually enlarged and the larva feeds lying in a curved position along the edge of the broken tissue. Feeding at the first site may continue for 2 or 3 days, or, if the hole extends to the leaf margin the larva may travel towards the leaf stalk to feed near the base of the leaf or may cross the leaf and begin to feed on the opposite edge.

When ready to moult the larva crawls to the edge of the upper side of a leaf, grips it with the prolegs and hangs the head and thorax over the edge. The thorax becomes distended, and the integument of the prothorax stretches until the annulation is no longer apparent. The skin ruptures along the mid-dorsal line of the thorax, along the epicranial suture and often along one arm of the frontal suture. The thorax then protrudes through the opening and the head is released when the chitinous intima of the stomodaeum is shed. The body is pushed forward and the legs are detached from the old skin and used for support, while the skin is worked backwards over the body segments and prolegs. The insect may rest a few minutes before the caudal segments are finally detached. The exuviae frequently remain attached to the leaf.

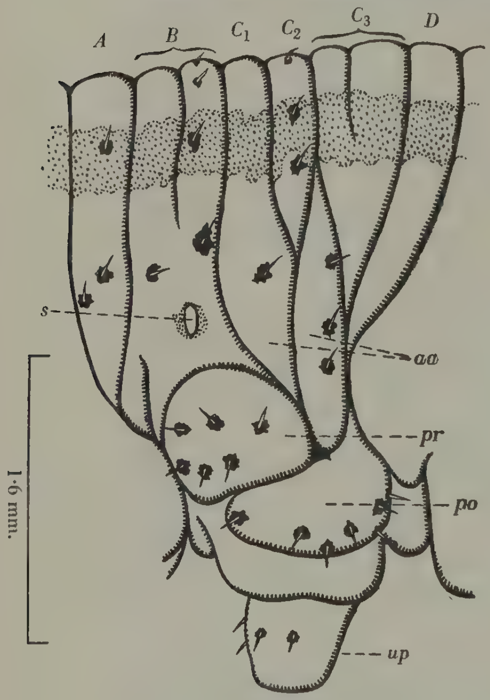


Text-fig. 1 A

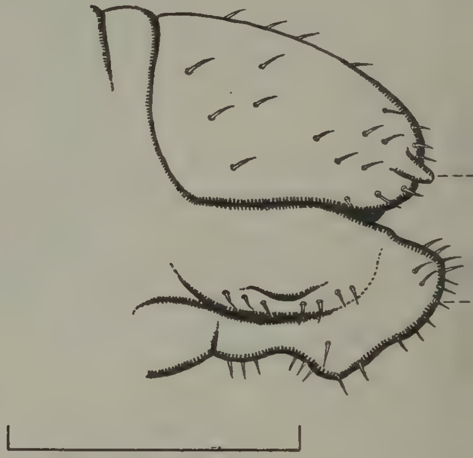
Text-fig. 1 A–G. *Holcocneme caeruleocarpa* Htg., larva. A, head lateral view. B, 6th segment lateral view showing annulation and setiferation. C, caudal segment showing pseudocerci (lateral view). D, caudal segment showing pseudocerci (dorsal view). E, labrum ventral view. F, maxilla. G, antenna. *o.* = ocellus; *a.* = antenna; *c.* = clypeus; *l.* = labrum; *m.* = mandible; *mx* = maxilla; *mx.p.* = maxillary palp; *l.p.* = labial palp; *s.* = spiracle; *a.a.* = alar area; *pr.* = pre-epipleurite; *po.* = post-epipleurite; *up.* = proleg; *psc.* = pseudocercus; *sa.l.* = subanal lobe; *ga.* = galea; *lac.* = lacinia.

In the second instar the larva is dark green and rather shining with the head black. Annulation and arrangement of setae are as occur in the first instar, but setae are inconspicuous unless the larva is examined with a lens. The second stadium lasts 3–4 days and the larva attains a length of about 10 mm. Larvae of the second instar bite through the leaf and feed lying along the edge as illustrated by Cameron (3) or may feed along the edge of the leaf. Usually they feed without the vigorous waving of the body frequently seen among gregarious nematine larvae.

In the third instar the larva is dark green dorsally and lighter green laterally and ventrally. The head is dark greenish brown and sparsely clothed with short dark setae, and short, inconspicuous setae also arise from faint dark spots on the alar area, epipleurites and certain of the annulets. The larvae in this instar feed along the margin of the leaf and attain a length of 14–15 mm. The third stadium lasts 2–4 days.

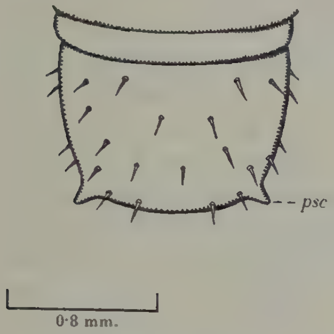


Text-fig. 1 B

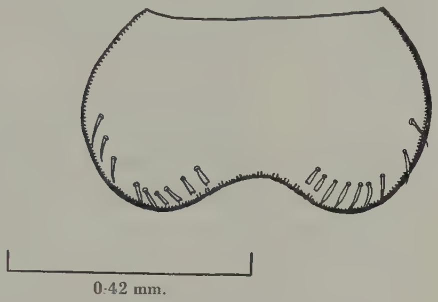


Text-fig. 1 C

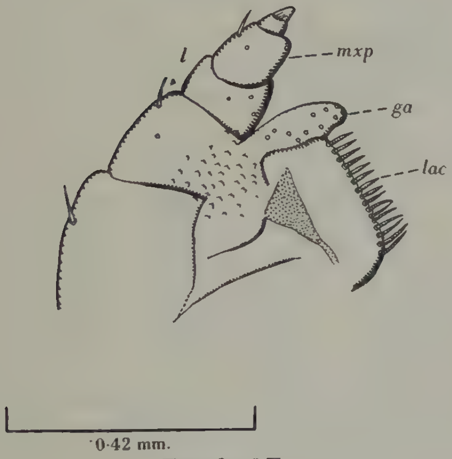
In the fourth instar the larvae are light or dark green with the head (Text-fig. 1 A) brownish or light, yellowish brown with darker coloration at the sides above the ocelli and on the vertex where there are also a few pale scattered setae. The ocelli are small and encircled by a black ring. The antennae are of the flattened disc type frequently met with in nematine larvae and, as the chitinous plates indicate, have four segments (Text-fig. 1 G). The frons is darker at the apex than at the base. The clypeus and labrum are light coloured, and the labrum is deeply emarginate (Text-fig. 1 E). The mandibles are dark brown at their tips and lighter brown at their bases. The maxillae are stout and fleshy and the maxillary palps are four-segmented. The form of the galea and lacinia is shown in Text-fig. 1 F. The labium is short and fleshy and bears short, three-segmented palps. On each side of the mid-dorsal line of the body are two inconspicuous longitudinal dark green bands which shade off into lighter areas which in turn are flanked by more distinct dorso-lateral dark green bands. Below the spiracular line the colour is pale green like the ventral surface. The legs are green, with the tarsal



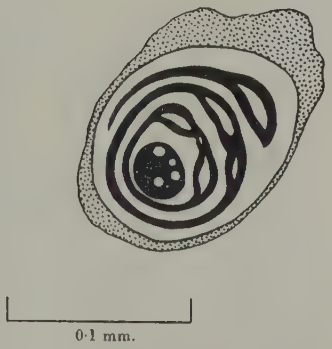
Text-fig. 1 D



Text-fig. 1 E



Text-fig. 1 F



Text-fig. 1 G

claws brown. The prolegs are pale green and the prominent and cylindrical pseudocerci are light brown and sometimes darker at the tips (Text-fig. 1 C and D). The annulation is sharply defined and even. A typical segment (Text-fig. 1 B) has six annulets (12) of which the second and fourth (*B* and C_2) bear a few scattered setae arising from dark bases. Setae arising from conspicuous dark spots also occur on the alar area and on the epipleurites. Larvae in the fourth instar are of two sizes. The smaller, and usually darker, are males and after feeding for 3–4 days they attain a length of about 18 mm. and leave the host plants for pupation. The larger larvae are females and after feeding for 2–4 days they moult. During the fourth stadium larvae feed voraciously; they cling closely to the leaf edges and their colour harmonises so well with that of the leaves that they are easily overlooked (Pl. XXXVII, fig. 2).

Only females have a fifth larval instar. The fifth stage lasts for about 4 days, during which the larvae attain a length of about 23–24 mm. They resemble larvae of the fourth instar and cling along the edges of the leaves when feeding. Under the name of *Nematus crassus* Fall.

Table I

H. caeruleocarpa

	Duration of stadia in days						
Nos.	1st stadia	2nd stadia	3rd stadia	4th stadia	5th stadia	Total	Sex
1	4	3	2	5	—	14	♂
2	3	4	4	4	4	19	♀
3	4	4	3	4	3	18	♀
4	4	3	3	4	—	14	♂
5	4	4	4	2	4	18	♀
6	4	4	3	3	4	18	♀
7	4	4	4	3	—	15	♂
8	4	4	3	3	—	14	♂
9	4	3	4	3	4	18	♀
10	5	4	2	4	3	18	♀
	3–5	3–4	2–4	2–4	3–4	14–19	

	Frons width in mm.				
Nos.	1st instar	2nd instar	3rd instar	4th instar	Sex
1	0·20	0·30	0·37	—	♂
2	0·20	0·29	0·37	0·55	♀
3	0·21	0·30	0·39	0·59	♀
4	0·19	0·31	0·38	—	♂
5	0·20	0·30	0·37	0·58	♀
6	0·20	0·30	0·40	0·57	♀
7	0·20	0·31	0·41	—	♂
8	0·19	0·30	0·40	—	♂
9	0·20	0·31	0·39	0·62	♀
10	0·21	0·30	0·41	0·58	♀
Average	0·20	0·30	0·39	0·58	
Growth ratio		1·5	1·3	1·5	Average 1·43

Brischke & Zaddach (2) figure larvae which for size, colour and attitudes are undoubtedly those of *Holcocneme caeruleocarpa*.

The larval stage of *H. caeruleocarpa* occupies 14–19 days during May and early June. Details given in Table I regarding a batch of ten larvae that hatched on 28 May show that larval development is fairly regular. Each stadium varied from 2 to 5 days. Males had four stadia and matured in 14–15 days and females became mature in the fifth stadium, 18–19 days after hatching. The growth ratio of these larvae was 1·43.

Construction of the cocoon and pupation

Fully fed larvae leave the host plants and penetrate 2–3 in. into the soil. They construct large dark elongate cocoons with soil particles incorporated in the outer layers of coarse silken strands. The inner layer consists of a secretion of saliva that hardens and becomes parchment-like. The cocoons are 12–16 mm. long, and the saw-flies escape through a circular hole cut in one end with the mandibles.

Adults from the first generation of larvae emerge after 21–27 days in the cocoon. The second generation of larvae hibernate in the cocoons and emerge after 9 or 10 months. Occasionally some members of the second generation emerge after 3–4 weeks in the cocoons and give rise to a third generation in the early autumn.

The annual cycle

H. caeruleocarpa is usually bivoltine in the north-west of England, but in favourable seasons there may be a partial third generation.

Adults emerge in spring over a period of 4–5 weeks, and as a result it is difficult to separate the generations in the field. From June to early August adults and larvae may be found on the host plants, while the insect is present in some form from April to October. An examination of collected material indicates that adults are numerous from 15 to 25 May, and during July and August, with July as the period of maximum occurrence. Under normal conditions adults that emerge in May produce larvae which become fully fed about a month later, and give rise to adults of the second generation in July. Larvae from the July adults become fully fed in August and generally pass into hibernation. In some seasons adults that emerge early in May produce a generation of adults which oviposits towards the end of July. The second generation of larvae mature by mid-August and produce a third generation of adults in early September. Larvae from this generation feed until early October with laggard larvae from the second generation.

Parthenogenesis

Arrhenotokous parthenogenesis, as noted by Enslin (4), occurs in *H. caeruleocarpa*, and eggs from virgin females produce males. In this the species resembles many species of *Pteronidea* and *Amauronematus* (14).

Males and females occur in approximately equal numbers in the field though males appear to be more numerous in July, possibly because inclement weather may cause females of the first generation to lay eggs before mating.

Distribution

Holcocneme caeruleocarpa appears to be common in the north-west of England, and is abundant in commercial willow beds. Perkins does not list the species from Devonshire, which suggests that it is much less common there than in the north. Morice (16) states that he has not taken it in the south-east of England, though it occurs in the London district and in Hertfordshire (R.B.B.). Cameron (3) states that while it appears common in England he has not taken it in Scotland. These observations suggest that although *H. caeruleocarpa* is common in parts of Britain, it is not generally distributed and appears to be confined mainly to the northern parts of England and, perhaps, south Scotland.

BIONOMICS OF *HOLCOCNEME LUCIDA* PANZ.

H. lucida occurs on wild and cultivated species of *Prunus*, but larvae are rarely so numerous as to cause appreciable injury to the foliage. It is a rather large, handsome saw-fly (Pl. XXXVII, fig. 3) with a distinctly tapering body and is mainly shining black with a broad reddish band across the abdomen, reddish tegulae and legs; the antennae are long, slender and dark-coloured; the wings are hyaline with the stigma and veins dark; and the tibiae have two stout spurs. Adults are on the wing from mid-April to mid-May, and may be found among the blossoms and unfolding leaves of plum, damson and blackthorn. Perkins (19) records *Holcocneme lucida* from *Crataegus* but the writer has taken neither larvae nor adults on this host.

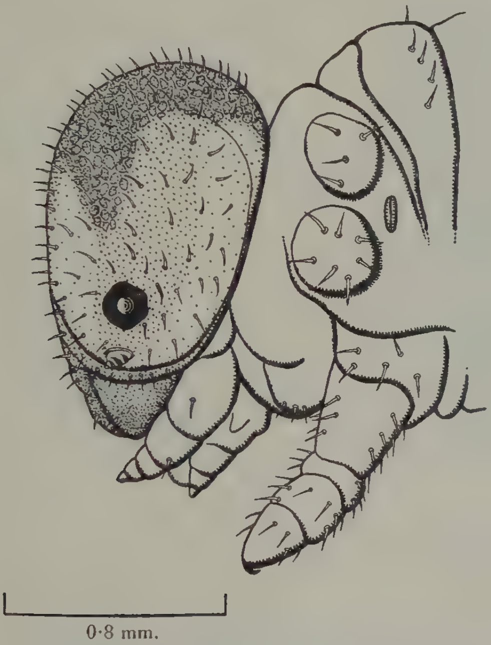
Oviposition and incubation

At oviposition the female makes a shallow longitudinal incision in the surface tissue of a vein on the under side of a leaf. The egg (Pl. XXXVIII, fig. 5) is laid along the incision and held in place by the edges of the tissue. In appearance and position eggs of *H. lucida* resemble those of *Pteronidea ribesii* Scop. (13) but are longer and taper distinctly at both ends. Usually only one egg occurs on a leaf but occasionally two may be found.

Eggs laid on 9 May hatched 18–19 May; eggs laid on 17 May hatched on 24 May, and eggs laid on 20 May hatched on 30 May. Thus the incubation period for eggs under observation varied from 7 to 10 days.

Larval stage

The larva emerges on the under side of the leaf and generally begins to feed at a short distance from the shrivelled chorion (Pl. XXXVIII, fig. 6). It bites a small hole in the leaf, then gradually enlarges the hole and lies in a curved position along its edge. Occasionally the tip of the body is raised above the edge of the hole or slightly curled on the under side of the leaf. The larva continues feeding and the hole becomes larger until it extends to the leaf margin. The larva may then continue feeding along the edge of the leaf, or may migrate to another leaf and feed along its edge. When feeding the larva remains almost motionless except for

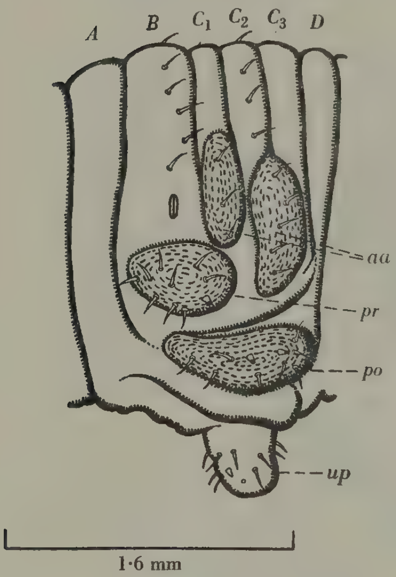


Text-fig. 2 A

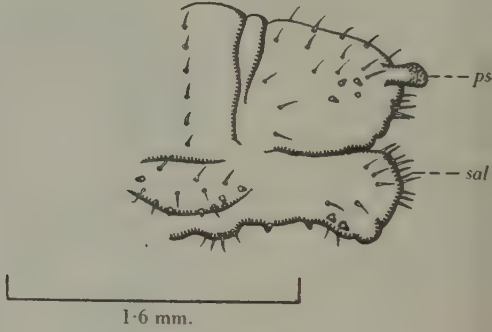
Text-fig. 2 A–G. *Holcocneme lucida* Panz., larva. A, head lateral view. B, 6th segment lateral view. C, caudal segment (lateral). D, caudal segment (dorsal). E, labrum ventral view. F, maxilla. G, antenna. (Lettering as in Text-fig. 1.)

rhythmic movements of the head and periodic voiding of frass. When disturbed it raises the abdomen and performs the rapid waving movements characteristic of gregarious saw-fly larvae.

In the first instar the larva is pale leaf green with a large black head and slender body slightly swollen at the tip. The caudal tergum is dark and bears short black pseudocerci. The thoracic legs are dark; the prolegs are pale green and, as is usual in *Nematini*, occur on segments 5–10 and 13.



Text-fig. 2 B

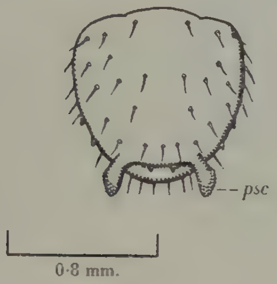


Text-fig. 2 C

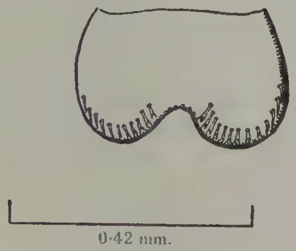
In the second instar the larva is pale green with a dark mid-dorsal band and the head black. The caudal tergum and pseudocerci are black, and small clusters of short dark setae with black bases occur on the epipleurites. The abdominal segments have six annulets, *A*, *B*, C_1, C_2, C_3 and *D*, of which *B* and C_2 bear short dark setae.

In the third larval instar the head is dark brown and covered with short dark setae. The body is pale green with a dark caudal plate and a row of small dark areas on the epipleurites. The short black pseudocerci are swollen and conspicuous; the legs are dark and bear black tarsal claws; and the dark setae are distinct on annulets *B* and C_2, and on the alar areas, epipleurites and prolegs. Larvae in the third instar measure 10–12 mm.

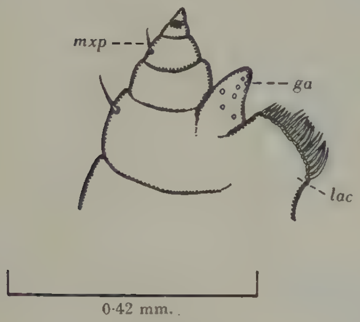
In the fourth larval instar the head (Text-fig. 2 A) is light, yellowish brown, with the frons dark brown, and dark brown areas extending along the epicranial suture and from the ocelli towards the vertex. In *Holcocneme lucida* the vertex, frons and genae are densely covered with short pale setae, whereas corresponding areas on the head of the larva of *H. caeruleocarpa* bear only a few scattered setae. The ocelli are small and set in black circles. The antennae (Text-fig. 2 G) have four segments, and are slightly larger and more prominent than those of larvae of *H. caeruleocarpa*. The clypeus and labrum are pale along their margins, and the labrum (Text-fig. 2 E) is deeply emarginate. The mandibles are pale with the teeth densely sclerotized. The maxillae (Text-fig. 2 F) are fleshy and have four-segmented palpi. The labium is prominent and fleshy, and the three-segmented labial palpi are short and slender.



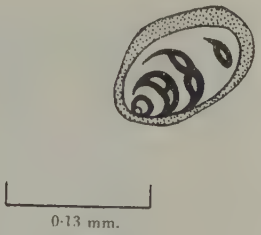
Text-fig. 2 D



Text-fig. 2 E



Text-fig. 2 F



Text-fig. 2 G

The body is rather bluish green dorsally and light grass green ventrally, the colours meeting along the spiracular line. Scattered setae occur on annulets B and C_2 of the segments and on the alar areas, the epipleurites and the prolegs (Text-fig. 2 B), but they are short and colourless, and rather difficult to distinguish. The thoracic legs are translucent pale green with the tarsal claws light brown, and the prolegs are pale green. The spiracles are small and inconspicuous. The epiproct is light yellowish brown, and the conspicuous pseudocerci are brown and dilated at the apex (Text-fig. 2 C and D). The suranal lobe bears a few short spiny processes and these, together with the pseudocerci, are of great value in distinguishing larvae of *H. lucida*. Larvae in the fourth instar measure 14–16 mm.

Males become mature in the fourth instar and enter the soil for hibernation and pupation. Females undergo a fourth ecdysis and have a fifth feeding instar. Larvae in the fifth instar (Pl. XXXVIII, fig. 6) are similar in appearance to those in the fourth but are 18–20 mm. long.

Table II gives details concerning the number and duration of the larval stadia of *H. lucida* and measured frons' widths in successive instars. Larvae of *H. lucida* kept under observation became fully fed in the period from 29 May to 28 June and after the latter date larvae were not taken in the field.

Table II

H. lucida

	Duration of stadia in days						
Nos.	1st stadia	2nd stadia	3rd stadia	4th stadia	5th stadia	Period	Sex
1	5	5	4	5	—	19	♂
2	4	4	3	3	—	14	♂
3	4	4	3	2	4	17	♀
4	4	3	2	3	4	16	♀
5	4	3	3	2	5	17	♀
6	3	2	2	4	—	11	♂
7	3	2	2	3	4	14	♀
8	3	3	2	3	3	14	♀
9	2	2	2	2 killed			♀
10	3 died						
	2·5	2·5	2·4	2·5	3·5		

	Frons width in mm.				
Nos.	1st instar	2nd instar	3rd instar	4th instar	Sex
1	0·237	0·312	0·387	—	♂
2	0·237	0·325	0·415	—	♂
3	0·255	0·325	0·425	0·575	♀
4	0·262	0·325	0·455	0·587	♀
5	0·262	0·325	0·470	0·612	♀
6	0·268	0·350	0·462	—	♂
7	0·250	0·350	0·462	0·550	♀
8	0·262	0·342	0·462	0·562	♀
9	0·255	0·320	0·455	—	—
10	0·250	—	—	—	—
Average	0·254	0·332	0·442	0·577	
Growth ratio		1·3	1·33	1·29	Average 1·3

Construction of the cocoon and pupation

Mature larvae leave the foliage of the host plant and construct cocoons in the top 3 in. of soil. The cocoons are of tough, parchment-like substance, oval in shape and very dark brown in colour, and are 10–12 mm. long. Larvae entering the soil in May or June remain in the cocoons as prepupae until the following March or April when pupation takes place.

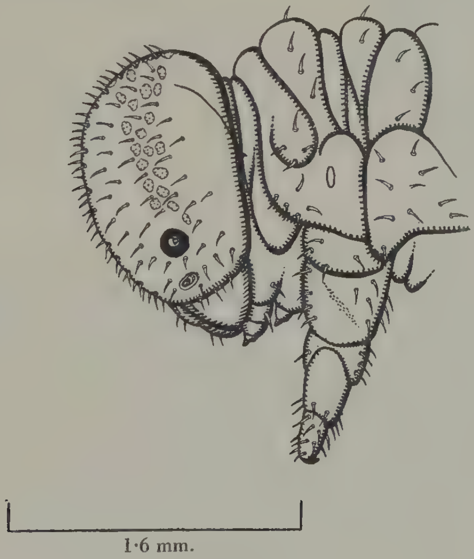
Annual cycle and parthenogenesis

Holcocneme lucida Panz. is univoltine. The species has arrhenotokous parthenogenesis and males and females appear in about equal numbers in the localities where the insects occur.

ON *HOLCOCNEME CRASSA* FALL.

Host plants, distribution and flight period

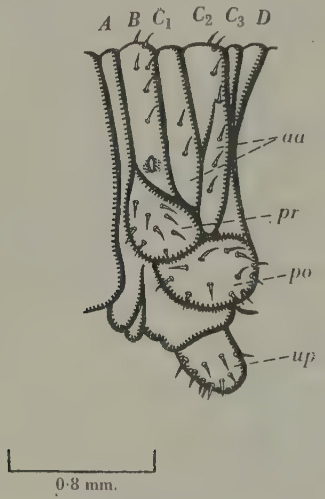
Little appears to be known of the biology and immature stages of this species. Zaddach (2) regarded it as a variety of *H. caeruleocarpa* Htg. Cameron (3) discussed the confusion that had long existed with regard



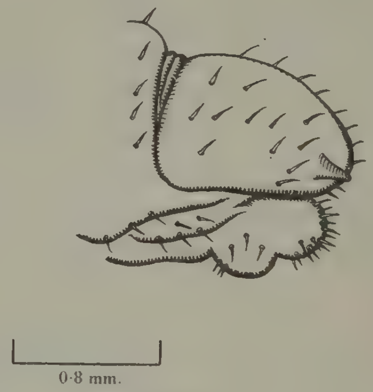
Text-fig. 3 A

Text-fig. 3 A–G. *Holcocneme crassa* Fall., larva. A, head lateral view. B, 6th segment lateral view. C, caudal segment (lateral). D, caudal segment (dorsal). E, labrum ventral view. F, maxilla. G, antenna. (Lettering as in Text-fig. 1.)

to both species and stated that he considered them distinct. Cameron thought *crassa* was "a northern species in Britain", since he obtained it in June on aspens at Braemar and Kingussie. Enslin (4) considered the two species distinct, but stated that the larva of *crassa* was not known with certainty. Heinrich (5) reared *H. crassa* from *Rumex obtusifolius* L. and gave a brief description of the larva. Perkins (19) obtained larvae of *Holcocneme crassa* from *Salix* in Devon, and Mr Benson, who reared the species from a larva found on *Betula*, has also verified Heinrich's observation by rearing the species from *Rumex* in Hertfordshire.



Text-fig. 3 B



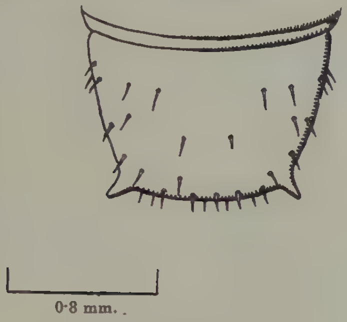
Text-fig. 3 C

Holcocneme crassa is widely distributed in Britain, and the British Museum collection contains specimens from Hampshire, Suffolk, Hertfordshire, Cambridgeshire, Surrey and Buckingham. Data on these specimens indicate that the flight period extends over May, June and July, but little is known of the annual cycle and nature of parthenogenesis.

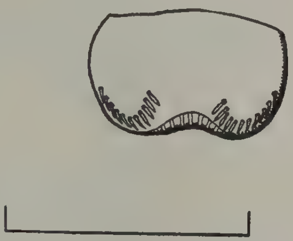
The following description and Text-fig. 3 were made from mature larvae reared on *Rumex* by Mr Benson.

The larva of Holcocneme crassa *Fall.*

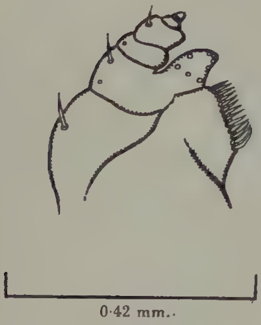
The head (Text-fig. 3 A) is greenish and sparsely clothed with short pale setae as in *H. caeruleocarpa*. Light brown streaks consisting of minute pigment spots extend from above the ocelli towards the vertex and along the epicranial suture. The ocelli are ringed by a narrow black or dark brown circle. The antennae are four-segmented and of the flattened disc type with a slightly protruding apical segment (Text-fig. 3 G). The frons is entirely pale; the trophi are pale, and the apex of the mandibles brown. The body is green, the dorsal surface being darker than the ventral surface. The spiracles are small and brown. The legs are green and clothed sparsely with short, pale setae, and the tarsal claws are brown. As in other nematine larvae, prolegs occur on segments 5–10 and 13 and bear scattered pale setae. The annulation is similar to that of *H. caeruleocarpa* and the epipleurites are distinct and protruding. A typical body segment (Text-fig. 3 B) has six annulets A, B, C_1, C_2, C_3 and D. Setae occur on B and C_2, on the alar areas below C, and on the pre-epipleurites and the post-epipleurites. The caudal segment (Text-fig. 3 C and D) is clothed with long setae, especially on the tergum and on the subanal lobe.



Text-fig. 3 D



Text-fig. 3 E



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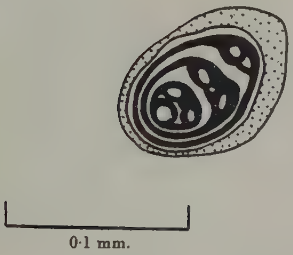


Text-fig. 3 G

The pseudocerci are prominent and acuminate, thus differing in shape from those of both *H. caeruleocarpa* and *H. lucida*.

BIONOMICS OF *HOLCOCNEME ERICHSONI* HTG.

One of the first accounts of the biology of *H. erichsoni* Htg. (17), the large larch saw-fly, in England is that of MacDougall (9) who, in 1906, gave a résumé of what was known of this species in European and American literature. In 1908 Hewitt (6) supplemented MacDougall's account with biological details that he had accumulated while studying the epidemic occurrence of the species in the Lake District of England. Subsequently Mangan (11), MacDougall (10), and Hewitt (7) made further contributions to the knowledge of *H. erichsoni*, and it is from these sources and the reports (1) published by the Board of Agriculture (Intelligence Division) that the following summary has been compiled.

Flight period

Adults begin to emerge from the cocoons towards the end of April and emergence continues until the end of July, the flight period being, therefore, 3 months. The period of maximum occurrence is the end of May and the beginning of June (Hewitt (6)).

Oviposition and period of incubation

The females lay their eggs in incisions in the new green growth at the tips of the shoots, about thirty eggs occurring in a shoot. The insect takes 3–6 min. to make an incision and lay the egg within it. The eggs are vitreous white in colour, elongate oval in shape, and about 1·5 mm. long. The shoots in which eggs have been laid tend to curl as growth proceeds, and they may wither and turn brown as a result of the wounds made at oviposition (Hewitt). The swelling of the eggs during incubation forces the sides of the incisions apart so that the eggs are partly visible in their pockets. The period of incubation is 8–10 days.

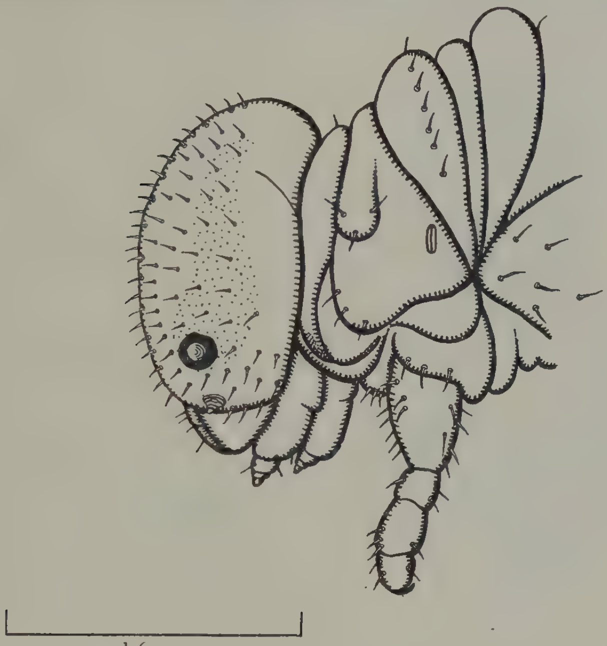
The larva

Since numbers of eggs occur together, newly hatched larvae feed gregariously and soon defoliate the shoots around the egg sites. They then move to neighbouring shoots to feed, and masses of half-grown larvae may be seen at the tips of defoliated shoots.

Hewitt (7) gives the following description of the larvae of the first instar:

"The newly-hatched larvae are greenish white and measure 2 mm. in length. The head is abnormally large and at first similar in colour to the body with brownish black eyes and brown jaws. By the end of twenty-four hours, the head has become sepia-brown in colour and the body assumes a greenish appearance owing to the presence of food in the intestine."

The mature larva (Text-fig. 4) has the head black or dark brown, and the body greyish green dorsally and pale green ventrally. In general form and colour the head bears a close resemblance to that of *H. lucida.* It is rather thickly set with pale setae and has streaks of darker colour extending from the ocelli towards the vertex which is also dark. The ocelli are surrounded by narrow dark brown rings; the antennae (Text-fig. 4 G) are pale, and, like those of larvae of *H. caeruleocarpa*, have the three basal segments flat and the apical segment conical. The frons is dark brown at the apex, and the clypeus is pale with a dark streak along the base, and the labrum (Text-fig. 4 E) is pale and emarginate. The mandibles are pale yellow with the teeth slightly darker. The maxillae (Text-fig. 4 F) are pale and fleshy and the palps are four-segmented.

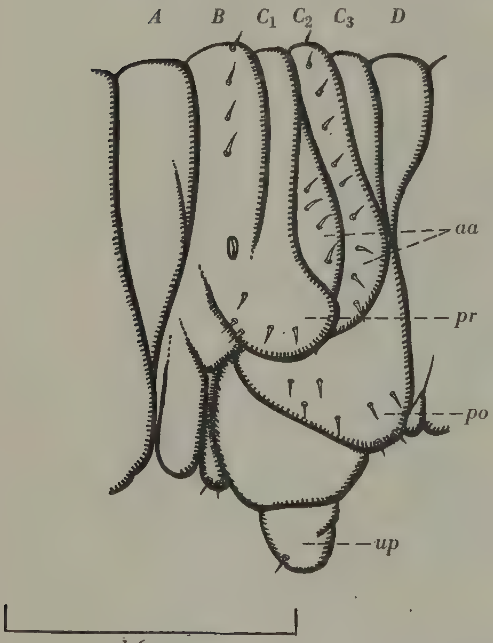


Text-fig. 4 A

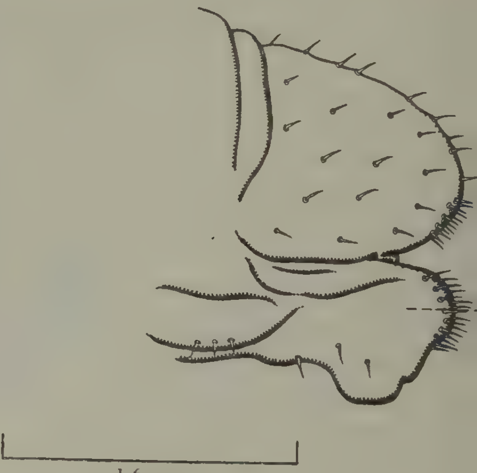
Text-fig. 4 A–G. *Holcocneme erichsoni* Htg., larva. A, head lateral view. B, 6th segment lateral view. C, caudal segment (lateral). D, caudal segment (dorsal). E, labrum ventral view. F, maxilla. G, antenna. (Lettering as in Text-fig. 1.)

The labium protrudes, as in *H. lucida*, and the labial palps are short, fleshy and three-segmented.

The annulation (Text-fig. 4 B) of the body segments is fine and even. Setae are short and colourless, and occur fairly thickly on the alar area and the epipleurites. Each typical segment has six annulets, *A*, *B*, C_1, C_2, C_3 and *D*; *B* and C_2 bear setae that arise from protruding colourless, raised bases (Text-fig. 4 B). The legs are pale green and the tarsal claws light yellowish brown, and prolegs occur on segments 5–10 and 13. The caudal prolegs are rather small and there are no pseudocerci, but the epiproct is closely studded with short colourless setae arising from slightly raised bases (Text-fig. 4 C and D). (Author's description from Hewitt's English material.)



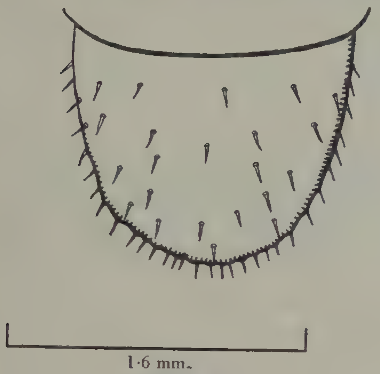
Text-fig. 4 B



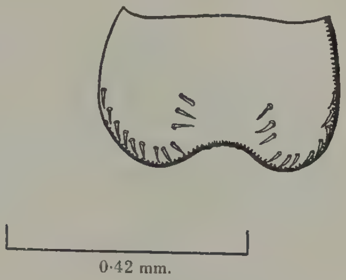
Text-fig. 4 C

Data collected by Hewitt in England (6) show that the larvae have six stadia before reaching maturity. Packard (18) stated that in America there appeared to be four stadia; but subsequently Hewitt (7) recorded that *H. erichsoni* had five larval stadia in Canada and pointed out that Packard might have missed a stadium since there were other errors in his account. It is, however, difficult to account for the difference between the number of larval stadia in *H. erichsoni* in England and Canada. There is, unfortunately, no information regarding the sex of the Canadian insects, but from the observations of others (MacDougall and Mangan) it seems that the English larvae were females.

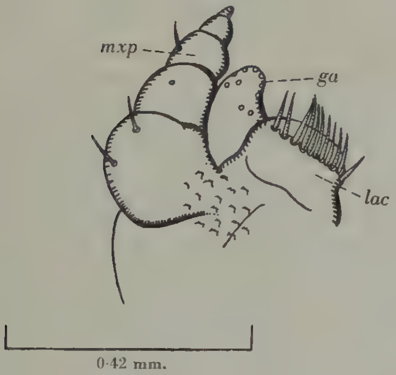
Observations on *H. lucida* and *H. caeruleocarpa* show that differences in the number of larval stadia are directly associated with sex, females having one more stadium than males. It is possible that Hewitt's Canadian larvae were males and, therefore, may have had one ecdysis less than the English larvae which appear to have been females.



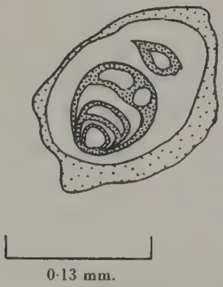
Text-fig. 4 D



Text-fig. 4 E



Text-fig. 4 F



Text-fig. 4 G

The annual cycle

According to Hewitt and others, *H. erichsoni* is univoltine. The emergence period is protracted, and some imagines emerge in late June and in July when the progeny of the early adults are reaching maturity.

Hewitt suggests that this seems to indicate that there is more than one generation in the year, but he states definitely that in his rearing experiments he failed to get emergences from larvae of the current year. The Board of Agriculture (1) publishes data that indicate that some individuals remain in the cocoons through two winters, a phenomenon that has been observed in certain other univoltine species (15).

SUMMARY

Four representatives of the genus *Holcocneme* Kon. occur more or less commonly in Britain.

H. caeruleocarpa Htg. breeds on *Salix* and *Populus*. It oviposits in pocket-shaped incisions in the leaves, and the eggs have an incubation period of 8–9 days. The young larvae are dark with the head black, older larvae are green with the head brown. A typical body segment has six annulets with dorsal setae on the second and fourth and the caudal segment has prominent cylindrical pseudocerci rounded at the apex. Females have five and males have four larval stadia, and pupation takes place in dark brown cocoons in the soil. There are two or three generations during the year, and the insects pass the winter in the cocoons. Parthenogenetic reproduction produces males.

Holcocneme lucida Panz. breeds on species of *Prunus* and lays its eggs in shallow incisions along the veins on the under surface of the leaves. The incubation period is 7–10 days. The larvae are yellowish green with the head brown. A typical body segment has six annulets of which the second and fourth are setiferous; the caudal segment bears prominent pseudocerci that are broader at the apex than at the base. Females have five larval stadia and males four. Pupation takes place in brown cocoons in the soil. The species is univoltine and parthenogenesis is arrhenotokous.

Holcocneme crassa Fall. is widely distributed in Britain and has been recorded as breeding on *Salix*, *Populus* and *Rumex*. The larvae are green with the head greenish brown and the annulation of the body segments is similar to that of *Holcocneme caeruleocarpa*. The setae are longer and rather denser than those of *H. caeruleocarpa* and the pseudocerci are acuminate.

The biology of *H. erichsoni* Htg. is described from the published accounts of MacDougall, Hewitt and Mangan. The females oviposit in the young shoots of larch; the larvae are greyish green, and though the annulation of the body segments is somewhat similar to that of *H. lucida*, the caudal segment has no pseudocerci and the arrangement of the setae differs from that of the other larvae described here. Females have six larval stadia. The tough brown cocoons are constructed at the surface of the soil. In Britain the species is thelytokous and parthenogenetic reproduction produces females.

The differences in the biology of the species and in the form and structure of their larval stages, together with differences in the structure of the adults and the nature of parthenogenesis suggest that the limits of the genus *Holcocneme* Kon. may not yet be adequately defined.

I am greatly indebted to Mr Benson for specimens of *Holcocneme crassa*; to Mrs Mary Miles, M.Sc., for assistance in the rearing of the larvae, and to Mr Morris Cohen, M.Sc., for help in the preparation of the text-figures.

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EXPLANATION OF PLATES XXXVII AND XXXVIII

PLATE XXXVII

Fig. 1. *Holcocneme caeruleocarpa* Htg., egg *in situ* in leaf of *Salix purpurea.* ×7.
Fig. 2. *H. caeruleocarpa* Htg., larva on leaf of *Salix.* ×7.
Fig. 3. *H. lucida* Panz., adult. ×4½.

PLATE XXXVIII

Fig. 4. *H. lucida* Panz., ovipositor. ×30.
Fig. 5. *H. lucida* Panz., egg in position on leaf of *Prunus.* ×8.
Fig. 6. *H. lucida* Panz., young larva at eclosion. ×8.
Fig. 7. *H. lucida* Panz., larva feeding on leaf of *Prunus.* ×3½.

(*Received* 3 *February* 1936)

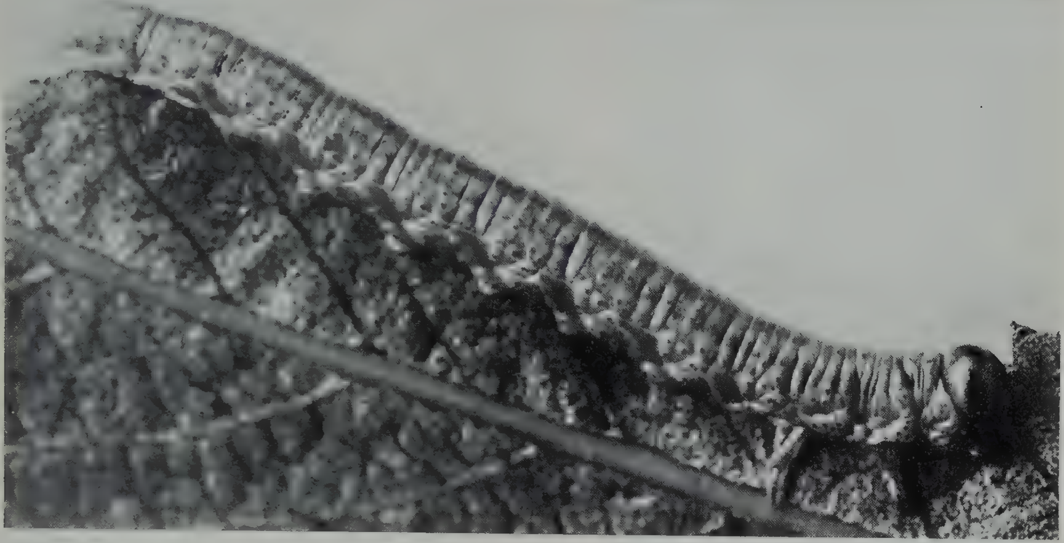

Fig. 2

Fig. 1

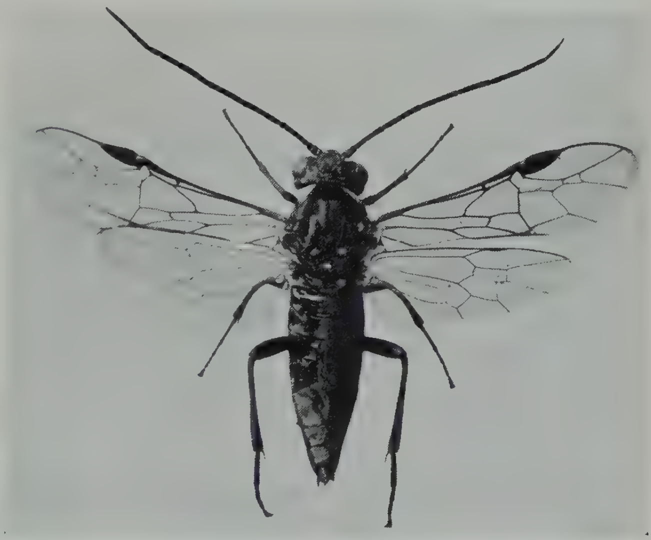

Fig. 3

MILES.—On the Biology of Certain Species of *Holcocneme* Kon. (Hymenoptera-symphyta)
(pp. 781–802)

EXPERIMENTS ON THE RESISTANCE OF THE FLOUR MOTH (*EPHESTIA KÜHNIELLA* ZELL.) TO ABNORMALLY HIGH TEMPERATURES

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(With 4 Text-figures)

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INTRODUCTION

THIS paper is primarily intended to be of practical value to those engaged in the control of the flour moth (*Ephestia kühniella* Zell.), but in addition some attention has been given to questions not directly concerned with control work.

At the beginning, the main object was to find the minimum exposures to various temperatures and humidities which were required to kill the various stages, and the experiments in which all or nearly all the individuals were killed were repeated several times. Later, an attempt was made to investigate some of the factors connected with death. The experiments are intended to give only a rough indication of the problems concerned, and much more needs to be done before the story is complete.

Since this paper is only a small contribution to the subject no discussion on the literature is included. This has already been admirably done by Buxton (5).

MATERIALS AND METHODS

The apparatus

The apparatus used is shown in Fig. 1. It consists of a large galvanized iron dustbin *A*, in which is fixed an iron pan *B*. The space between *A* and *B* is filled with water and heated by the batswing burner *C*. The bin is suitably covered with blankets *H* to provide insulation. The space over the water between the bin *A* and the pan *B* is covered with a sheet of aluminium, and the pan *B* is covered with two sheets of heavily waxed three-ply wood, in which are cut eight holes on a circle and one central hole. Supported in each of the eight holes by a rubber collar and hanging into the pan *B* are glass tubes *D*, having bags of muslin tied over the

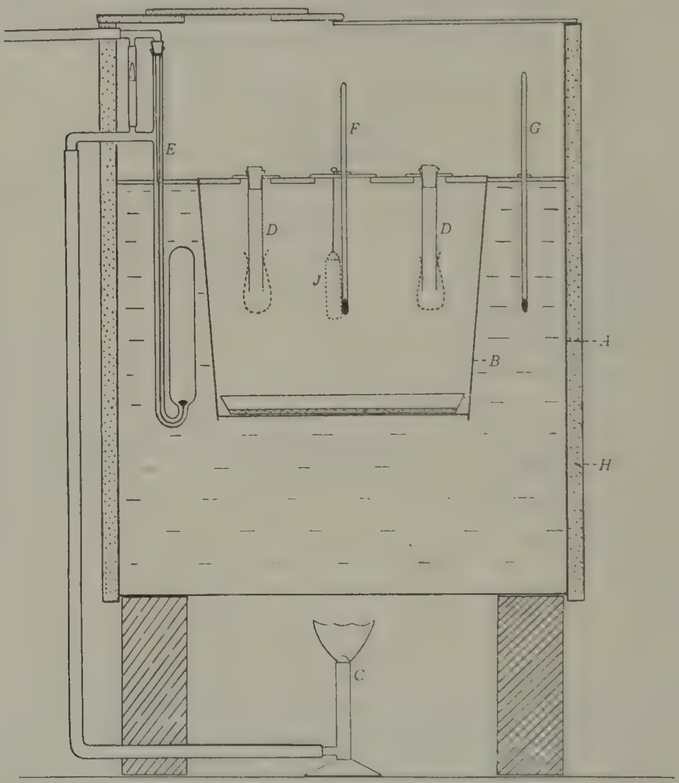

Fig. 1

lower ends. Each tube is fitted with a rubber bung. The insects to be heated are dropped down the tubes into the muslin bags, and the bungs are replaced.

The temperature of the water in the bin is controlled by the gas thermostat *E*, which consists essentially of a bulb of toluene and a column of mercury, which on rising partially cuts off the gas supply to the burner *C*.

Covering the bottom of the pan *B* is a dish containing a saturated salt solution to control the humidity.

There is one thermometer *F* in the centre of the pan *B* and another *G* in the water jacket. A third was sometimes placed in one of the tubes *D*. The temperature of the water jacket was always 0·9–1·1° C. higher than the centre of the pan *B*. No variation in temperature has been noticed in the pan *B*.

The humidity was measured by a weighing hair hygrometer (Buxton (4), p. 436) in a muslin bag *J* suspended in the pan or in one of the tubes *D*.

In order to carry out experiments with different humidities at the same time special tubes have been used in place of the tubes *D*. They are slightly smaller than *D* and enclosed by a large tube, at the bottom of which is the appropriate saturated salt solution (Fig. 2). A disadvantage of these tubes is that they are too small to admit any hygrometer, and the humidity has to be estimated by keeping a hygrometer in a similar but larger tube. Another disadvantage is that a smaller quantity of material has to be used, and as there is only a small volume of air a constant humidity is not so easy to maintain.

The humidities have been obtained as follows:

1–3 per cent in tubes as shown in Fig. 2, with anhydrous calcium chloride.

8–10 per cent in the pan *B* with anhydrous calcium chloride (a lower humidity was not obtained owing to considerable leakage of air into the pan).

30 per cent in tubes as shown in Fig. 2 with sodium hydroxide (saturated solution).

70–75 per cent in the pan *B* and in the tubes (Fig. 2) with a saturated solution of sodium chloride.

90–95 per cent in the pan *B* and in the tubes (Fig. 2) with distilled water.

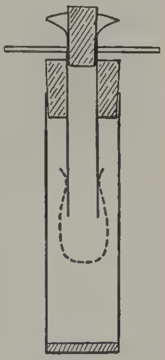

Fig. 2

As far as possible the temperatures were selected for each stage so as to allow about 1 hour's exposure for a total kill, in order to try and avoid any shock effect that might be due to sudden exposure to a high temperature. For example, eggs at 45° C. and 70 per cent relative humidity (R.H.) are not killed under 4 hours, while adults at this temperature and humidity are killed in 15 min. Even if the question of shock is not important in the case of short fatal exposures, experiments of less than 15 min. duration are not satisfactory, as during so short a time the insects probably never come into equilibrium with the temperature and humidity.

The insects were plunged direct into the given temperature, as it did not seem possible to give gradual exposures which would have the same value in all experiments.

For the main experiments the eggs were heated at 47° C. (116·5° F.), the larvae and pupae at 45° C. (113° F.) and the adults at 44° C. (111° F.).

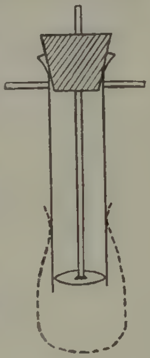

Fig. 3

Description of materials

As will be seen, it is necessary to pay particular attention to the history of the insects used. All the *E. kühniella* used in these experiments came originally from the same flour mill, and the main supply from which the "inbred stock" originated was obtained in 1930–1.

Inbred stock. Most of the experiments were carried out on this stock which had been kept in the insectaries for about 2½ years, and inbred continuously for about twelve generations. The progeny of each pair was kept separately, each culture being the result of brother and sister mating. There are probably several more or less distinct lines in this inbred stock, but an exact account of these has not been kept.

Normal stock. For comparison with the "inbred stock", material was obtained from the flour mill in the summer of 1933 and utilized before there had been one generation in the insectaries.

In 1931 a few experiments were carried out on stocks which were the ancestors of the inbred stock and had been more or less inbred for three generations. This material was used for the experiments shown in Table II under 8–10 per cent R.H. This is also called normal stock, as its reactions to heat in these and other experiments are similar to those of the normal stock obtained in 1933.

The system and conditions under which the inbred stock is reared in the insectaries has been described (Mansbridge(8)). The insectaries were usually at a temperature of about 18° C., though occasionally it fell to about 10° C. in the winter and reached about 28° C. in the summer.

Adult insects were obtained by keeping a number of pupae at 27° C. (81° F.) and removing the moths daily. The moths were always kept at 27° C. until needed for an experiment. In using the tube *D* for moths an addition had to be made to keep the moths in the muslin bag; a wooden rod was passed through the cork to the bottom of the tube, and a waxed paper disc at the lower end prevented the moths climbing back up the tube (Fig. 3).

Eggs were obtained by collecting 20–60 pairs of adult moths in copulation, and leaving them to lay for 12 or 24 hours at 27° C.; the eggs which were 0–12 hours or 0–24 hours old were then removed. The eggs from each pair of moths were divided into a number of equal lots (one control lot and usually four or five experimental lots). They were always kept at 27° C. until required.

Newly hatched larvae were removed from batches of eggs hatching at 27° C. and were never more than 12 hours old when used.

Feeding larvae, about half-grown, were picked out from cultures in the insectary. They were kept at least for 24 hours at 27° C. before an experiment, and during this time some died from bacterial disease. Only quite healthy larvae were used.

Last-stage larvae were taken from cultures in the insectary. They were kept for at least 24 hours at 27° C., and only healthy larvae were used. These larvae were full grown and could be distinguished from the feeding larvae by their larger size; in some cases they had started spinning cocoons. In the males the gonads could be clearly seen in the living larvae.

Prepupae were taken from batches of last-stage larvae which had been kept 1–4 days at 27° C. The prepupa is regarded as the stage about 24 hours before the shedding of the last larval skin. It is distinguished from the larvae by having lost all power of forward movement; it is shorter than the larva and often has a greenish hue.

Pupae of definite ages were obtained by keeping a large number of full-grown larvae at 27° C., and removing the pupae every 24 or 48 hours. Pupae were always kept at 27° C. until required.

Method of judging survival

All insects after the experiments were kept at 27° C. and approximately 70 per cent. R.H. (over a saturated solution of sodium chloride) and the survivors recorded as follows:

Eggs. The number of hatched larvae, found alive, the larvae being removed at least every 24 hours. To confirm the figures thus obtained the unhatched eggs were sometimes counted.

Newly hatched larvae. Those found crawling 24 hours after the experiment.

Other larvae (i.e. feeding and resting larvae). Those found crawling 2 days after the experiment.

Prepupae. The number of healthy pupae.

Pupae. The number of moths emerging.

Adults. Those found crawling 24 hours after the experiment (i.e. those making co-ordinated forward movements). "Crawling" is taken to mean a definite normal movement, as distinct from the spasmodic jerks and twitches of injured and moribund insects.

The survivors as recorded above have lived for a long time after the experiment. A few of the survivors from experiments on each stage have been kept; and in most cases these have given rise to a second generation.

Method of recording results

In recording the results of the experiments on eggs, the number of survivors is given as a percentage of the surviving controls. For instance, if 270 individuals were used (in each batch) and 230 survived in the controls and 125 in the treated lot, then the survivors are given as $\frac{125 \times 100}{230} = 54$ per cent. The hatch of the control batches of eggs varied between 50 and 90 per cent.

With other stages it was found unnecessary to record the survivors as a percentage of the surviving controls, as very seldom did more than two out of fifty of the controls die. Usually the control larvae, pupae and adults were in good condition, as only the healthiest insects were selected for experiment. By submitting all larvae to a temperature of 27° C. for 24 hours diseased individuals were easily detected because the rapid development of disease at this temperature caused them to turn black. Bacilli believed to be *B. thuringiensis* were known to infest the stocks from time to time, and it may be assumed that these were the most important pathological factor.

In all the tables the results have been expressed as the percentage which survived. Although less than 100 individuals were used in the experiments on adults and pupae, it was thought best to express all the results in the same way. The number of eggs used has usually been from 500 to 1000 in each batch, and in some cases many more. With larvae about 100 and not less than 50 have been used for each batch, with pupae about 50 and not less than 25, with adults about 25 and not less than 12.

DESCRIPTION OF EXPERIMENTS

In all stages of *E. kühniella* there appears to be great individual variation in the resistance to heat. This is most noticeable in the eggs and larvae.

Eggs

Below are given some of the factors which appear to affect the survival of the eggs at high temperatures:

(1) *Age.* Table I shows that there is a decrease in resistance with age at 45° C.

(2) *Humidity.* Table II shows that eggs are less resistant at high than at low humidities. From experiments to be referred to later, it is almost certain that eggs can remain cool in a low humidity at a high temperature by evaporating water.

(3) *Number of eggs heated together.* At a low humidity there is a relatively bigger hatch in a large batch of eggs treated together, than in a small batch of similar eggs. In the method employed the eggs may be treated in a heap, and frequently a mass of fifty or 100 eggs attached to one another are heated, or they may be lying scattered. Eggs in a heap probably have a better chance of keeping cool than if they are scattered (see p. 817).

(4) *Individual variation.* Table III shows that eggs from different pairs of moths have widely different degrees of resistance.

Table I

Eggs. To show changes in resistance with age, and a comparison between the resistance of "inbred" and "normal" stocks. Giving percentage hatch of eggs

Time of exposure at 45° C.	Inbred stock Age of eggs 1–24 hr.	24–48 hr.	48–72 hr.	72–96 hr.	Normal stock Age of eggs 1–24 hr.
70 % R.H.:					
1 hour	61·9	26·2	0·0	0·0	75·6
2 hours	24·4	2·1	0·0	0·0	56·6
95 % R.H.:					
1 hour	63·9	19·5	0·0	0·0	84·4
2 hours	37·0	6·6	0·0	0·0	60·9

(5) *History of material.* In Tables I, II and III there is seen to be a marked difference in resistance between the inbred and normal stocks. From Table III it is not possible to say whether the normal stock is

more or less resistant than the inbred stock, but it appears that there is a greater range of resistance, high and low, in the normal stock, and that it is less uniform than the inbred stock. Its chief interest is that it shows the great range of variability. From Table II it is seen that there is a small percentage of resistant individuals in the normal stock which survive exposures considerably higher than those which kill the inbred stock.

Table II

Eggs 1–24 *hours old. To show the effect of various relative humidities on resistance, and a comparison between the resistance of "normal" and "inbred" stocks. Giving the percentage hatch after exposure to* 47° C. *for various periods*

Time of exposure at 47° C. hours	Inbred stock (% R.H.)				Normal stock (% R.H.)		
	1–3	30	70	95	1–3	8–10	70
¼	55·8	55·8	41·1	31·4	73·7	—	55·7
½	47·0	47·3	20·1	6·2	30·4	47·1	15·9
1	9·2	10·6	0·0	0·0	22·1	21·9	0·0
1¼	0·0	8·2	0·0	—	4·3	5·5	—
1½	0·0	0·0	0·0	—	1·9	2·5	—
1¾	—	0·0	0·0	—	0·6	0·5	—
2	0·0	0·0	—	—	0·0	0·5	—
2¼	0·0	—	—	—	—	0·0	—

Table III

Eggs 1–24 *hours old. To show individual variation exposed at* 47° C., *and* 1 *and* 70 *per cent* R.H. *Each figure gives the percentage hatch of eggs from a separate pair*

Normal stock					Inbred stock		
70 % R.H.			1 % R.H.		70 % R.H.		1 % R.H.
Time of exposure at 47° C.							
¼ hr.	½ hr.		½ hr.	1 hr.	¼ hr.		½ hr.
42·0	0·0	0·0	24·7	20·4	53·4	45·6	32·2
42·1	0·0	22·7	4·2	23·5	89·5	54·9	36·4
0·0	3·0	12·9	8·1	0·0	69·1	28·7	34·6
68·7	12·0	7·9	37·4	1·7	82·1	22·6	36·0
91·3	99·5	1·9	—	0·0	59·6	38·9	—
0·0	0·4	0·4	—	0·0	—	32·5	—
74·7	53·9	0·5	—	—	—	—	—
65·4	1·0	0·0	—	—	—	—	—

Hatch of controls: normal stock = 50–80 %, inbred stock = 47–59 %.

(6) *Humidity of "after-treatment"*. Table IV shows that after eggs have been heated at a low humidity there is a much bigger survival if they are kept to hatch in a high humidity than in a low humidity. This

is owing to the ability of the eggs to lose water during heating at low humidities and to regain it again at normal temperatures (see pp. 815–817). It was thought that eggs might be unable to hatch at a low humidity, and to test this a batch of 900 newly laid eggs was kept over anhydrous calcium chloride (giving 1–3 per cent R.H.) at 27° C. 385 of these eggs hatched, and 78 per cent in the controls, making a hatch of 55 per cent of the surviving controls.

Table IV

Eggs 1–24 *hours old* (*inbred stock*). *Showing the effect of keeping eggs under various relative humidities* (*at* 27° C.) *after they have been heated at* 47° C. *and* 1–3 *per cent* R.H. *for various periods. Giving the percentage hatch*

Time of exposure at 47° C., 1–3 % R.H.	Relative humidity after treatment (%) 1–3	30	55	75	95
½ hour	0·0	11·3	—	39·4	49·3
1 hour	0·0	8·0	12·0	17·0	25·0

In order to test whether the humidity of pretreatment had any effect on the resistance of the eggs, batches were kept at high and low humidities before heating but no marked difference in resistance was found.

Eggs which were heated at exposures near which some survival took place, showed a more or less large number of unhatched eggs with well-formed embryos. In some cases larvae died while half out of the egg. These phenomena were particularly noticeable in short-period, low-humidity experiments, and may have been due to low water content in the egg. It was regularly noted that heated eggs took longer to hatch than the controls. This delay in hatching occurred in every experiment whatever the humidity or exposure. At 27° C. the delay amounts to from 24 to 36 hours, and at room temperatures from 2 to 5 days. This may have been due to the water content of the egg being lowered, and so the rate of development slowed down.

Larvae

Larvae at three different ages have been heated, *newly hatched feeding* and *last stage*. The experiments on larvae heated below the fatal point gave erratic results, but the exposures at which all were just killed were constant for each age. These fatal exposures are given in Table V. Newly hatched larvae were heated within 12 hours of hatching and before they had fed. Other newly hatched larvae were placed at 27°C. in the constant temperature room, and their resistance was tested at the following ages: 3, 6, 10, 14, 15, 17, 19, 20, 22, 23, 26, 27, 28, 29, 31 and 36 days old. Larvae from 3 to 19 days old (feeding larvae) were all killed by exposures between 22 and 28 min. at 45° C. Larvae 22–26 days old were more resistant, but were all killed by exposures of 40 min. Larvae of 27 days old and older were found to be much more resistant and a complete kill was only obtained by 1¾ hours' exposure. These larvae over 27 days old (at 27° C.) have been called last-stage larvae. They have finished feeding and in most cases have started to spin cocoons.

Table V

Larvae. Giving the exposures necessary for a total kill at 45° C. *and various relative humidities*

	Inbred stock			Normal stock
	1–3 % R.H.	70 % R.H.	95 % R.H.	70 % R.H.
Newly hatched larvae	25 min.	40 min.	25 min.	45 min.
Feeding larvae	40 ,,	40 ,,	20 ,,	35 ,,
Last-stage larvae	1¾ hours	1¾ hours	1¾ hours	2 hours

In most experiments half-grown feeding larvae and full-grown last-stage larvae have been used without regard to their exact ages.

In the last-stage larvae the sexes can be distinguished, but the results for the two sexes have not been given separately as little difference was found. In ten experiments in which males and females were heated simultaneously under the same conditions (522 males and 393 females) 22·8 per cent of the males survived and 20·3 per cent of the females. This small difference in resistance is not regarded as significant.

All larvae survive slightly better at low than at high humidities in short exposures. This suggests that they can remain cool for short periods at low humidities. As with the eggs, when large numbers of larvae were heated together at low humidities a bigger percentage have survived than when small numbers were heated.

Larvae which were just not killed often fail to cast their larval skin at pupation, but the deposition of chitin still takes place. Sometimes the pupa may only partly disengage itself from the larval skin, and remain bent; such pupae have generally died.

Larvae which survived 46° C. for 1 hour, although ready to pupate before heating, failed to pupate, some being alive 2 weeks afterwards (kept at 23° C. 80 per cent R.H.).

During heating at low humidities the larvae commonly ejected a brownish fluid from the anus and mouth, which often glued these parts down to the sides of the muslin bag.

A special strain of newly hatched and last-stage larvae, inbred for about nine generations and selected for high fertility, proved considerably less resistant than either the inbred or normal stocks.

Table VI shows experiments on prepupae. Survival was best at 70 per cent R.H. Nearly all the prepupae which were heated deposited brown chitin under their larval skin, and in most instances the larval skin remained uncast; sometimes it was only partly cast. The prepupae in this state did not die at once but remained showing some sign of life for 1 or 2 weeks. This seems to show that the cause of death is probably quite different to what occurs in any other stage. Here death is not due to the direct influence of heat but to an interference with moulting.

Table VI

Prepupae. To show the effect of various relative humidities on resistance at 45° C. Giving percentage survival

Time of exposure	Females (% R.H.)			Males (% R.H.)		
	1–3	70	95	1–3	70	95
35 min.	44	72	40	36	60	40
45 min.	16	50	10	14	50	20
1 hour	0	6	0	0	0	0
1¼ hours	—	0	—	—	0	—

Table VII shows experiments on pupae. Here in many cases there is an almost complete survival for an exposure of 1 hour at 45° C. This may be due to the fact that the pupae are less variable than the larvae.

Table VII

Pupae (inbred stock). To show changes in resistance with age at 45° C. 70 *per cent* R.H. *Giving percentage survival*

	Time of exposure in hours	Age (in days)			
		1–2	3–4	5–6	7–8
Females	1	96	100	96	54
	1¼	72	96	84	48
	1½	28	88	76	42
	2	0	32	0	0
	2¼	—	0	—	0
Males	1	84	84	88	94
	1¼	48	64	76	64
	1½	32	24	28	0
	1¾	4	4	0	—
	2	0	0	0	—

In Table VIII it is interesting to notice the effect of an exposure of 1½ hours at three different humidities. The biggest survival appears to be at the low humidity, which may mean that at that exposure the pupae can remain cool by loss of water.

Table VIII

Pupae (*males*) 3 *and* 4 *days old. To show the effect of varying relative humidities on resistance at* 45° C. *Giving percentage survival*

Time of exposure hours	1–3 % R.H.	70 % R.H.	95 % R.H.
1	100	100	96
1½	60	28	14
1¾	0	6	4
2	—	0	0

Table IX shows experiments on unpaired and paired adults, at 8 per cent R.H. Here, and in similar experiments at 75 per cent R.H., there is a decrease in resistance with age. The paired adults show a difference in resistance from the unpaired individuals, as after pairing and egg laying they are less resistant than those which have not paired. Unlike unpaired moths, in paired moths there appears to be no difference between the sexes.

Table IX

Adults (*unpaired and paired*). *Normal stock. To show changes in resistance with age. Giving exposures necessary for a total kill at* 45° C. 8 *per cent* R.H.

		Age (in days)				
		0–1 min.	1–2 min.	2–3 min.	4–6 min.	7–9 min.
Unpaired:	Females	17	20	15	8	7
	Males	11	9	8	6	5
Paired:	Females	(in cop.) 21	12	12	8	—
	Males	21	12	12	8	—

From Table X it appears that the females are more resistant than the males at low humidities, possibly because females are better able to cool themselves by evaporation, which may be due to their rather larger size.

Table X

Adults (*unpaired*), 1 *day old* (*normal stock*). *Showing the effect of various relative humidities on the time* (*in minutes*) *required for a complete kill at* 44 *and* 45° C.

		15 % R.H. min.	37 % R.H. min.	70 % R.H. min.
Females:	44° C.	60	75	30
	45° C.	17	30	15
Males:	44° C.	35	75	35
	45° C.	10	30	10

Fig. 4 summarizes approximately the differences in resistance to a temperature of 45° C. and 8 per cent R.H. shown throughout the life cycle.

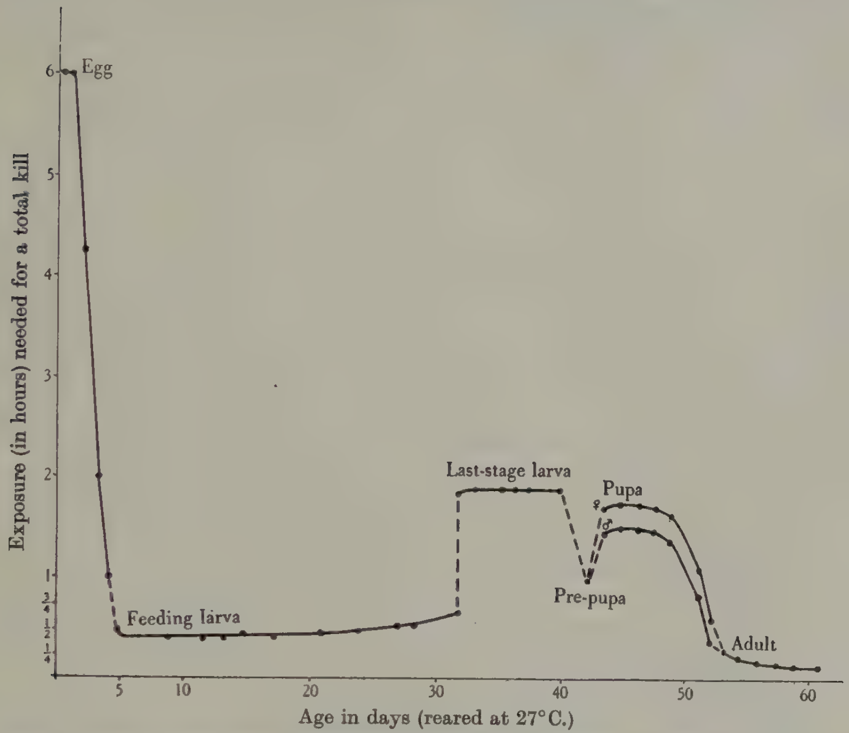

Fig. 4. Graph showing varying resistance to heat of the flour moth at 45° C. 8 per cent. R.H. throughout its life cycle. Normal stock. See table XIII for comparison with other humidities.

EXPERIMENTS ON THE LOSS OF WATER FROM EGGS EXPOSED TO HIGH TEMPERATURES AND LOW HUMIDITIES

As it seemed likely from the foregoing results that *Ephestia* eggs were able to cool themselves by evaporating water during heating at low humidities, some experiments were set up to find out how much water the eggs could lose under varying conditions. It was assumed that most of the weight lost by eggs in a dry atmosphere was due to loss of water. Table XI summarizes some of these experiments.

The method of calculating the extent to which an insect can cool itself by evaporating water is taken from Mellanby (9). The constants are from Preston (12); they cannot be taken as applying exactly to insects but they should give some idea as to the order of the figures concerned.

Table XI

Eggs (inbred stock). Showing changes in weight at different humidities. Also the loss of weight during heating at low humidities and its regain in a moist atmosphere at 27° C.

No. of exp. ...	1	2	3	4	5	6	7	8	9	10	11
Temp. °C.	27	27	27	47	47	43	47	47	43	43	43
Time of exposure hours	—	—	—	1	3	2½	½	½	12	1	6
R.H. of heating %	—	—	—	1–3	1–3	1–3	1–3	30	1	1	1
R.H. of "after-treatment" %	1	60	95	95	95	95	—	—	—	—	—
Hatch %	43	69	57	40	0·0	22	12	50	0·0	90	19
Percentage gain (+) or loss (−) on original weight:											
Immediately after heating	—	—	—	−11·6	−35·3	−21·7	−25·0	−25·0	−36·0	−16·0	−18·0
After 4½ hours	− 5·0	− 2·4	+0·3	−6·9	−35·0	−19·3	—	—	—	—	—
After 15 hours	−20·8	− 5·7	+1·8	−6·1	−30·9	−17·9	—	—	—	—	—
After 45 hours	−30·4	−10·5	+1·1	−5·5	−31·4	—	—	—	—	—	—
After 65 hours	−41·2	−16·7	—	—	−31·1	−17·0	—	—	—	—	—
No. of eggs in experiment	900	1000	1000	4400	1000	1620	648	500	835	835	2000

N.B. 1000 fresh eggs weigh approximately 0·0300 g.

For every square centimetre of surface and for every degree C. that the temperature is lower than the air, an insect will absorb 0·00025 calorie per sec. This will mean approximately 1 calorie per hour.

For an insect to maintain its body at a lower temperature than the air, the heat absorbed must be got rid of, and it is assumed that this is done by evaporating water.

Now at 47° C. every gram of water evaporated requires almost 600 calories. Therefore, provided that the cooling mechanism of an insect is very efficient, for every calorie of heat absorbed, about 2 mg. of water will have to be evaporated if the body is to maintain a constant temperature.

An *E. kühniella* egg weighs about 0·030 mg., and has a surface area of about 0·0043 sq. cm. (length 0·57 mm., diameter 0·30 mm.).[1] If it is to keep itself 1° C. cooler than the air for half an hour, and if the air is at 47° C. and the egg at 46° C. it will absorb about 0·0021 calorie, which will evaporate 0·0035 mg. of water. This is about 11 per cent of the weight of the egg. The experiments (Table XI, Nos. 4 and 6) show that in a mass the eggs can lose about 12 per cent of their weight and 40 per cent of the eggs hatch, or lose 22 per cent of their weight and 22 per cent of the eggs hatch.

---

[1] Formula for calculating the surface areas of an egg:

$a = 2{\cdot}22d\sqrt{D^2 - d^2}$, when d = short diameter, a = area, and D = long diameter.

From Table XI we can calculate how much of the water lost can be regained. Exp. 4 shows that nearly 40 per cent of the water lost is regained within the first 4½ hours. This water has been regained by live eggs, for Exp. 5 shows that dead dried eggs take up little or no water during the first 4½ hours' exposure to 95 per cent R.H. As 40 per cent of the eggs are found to hatch and 4·7 per cent of the original weight is regained, then each of the surviving eggs regains on an average $\frac{100}{40} \times 4{\cdot}7 = 11{\cdot}75$ per cent of its weight. In Exp. 6 it is seen that more than one-tenth of the water lost is regained within the first 4½ hours. As 22 per cent of the eggs in this experiment are found to hatch, and 2·4 per cent of the original weight is regained, then each of the surviving eggs regains in weight on an average 10·80 per cent of its weight.

In these two experiments it is unlikely that more water is absorbed in the first 4½ hours than was originally in the egg. As a check to this it is seen in Exp. 3, Table XI, with unheated eggs, that there is an increase of only 0·3 per cent in weight after exposure for 4½ hours to 95 per cent R.H.

These experiments show that eggs can lose at least 10 *per cent of their weight in water and thus cool themselves about* 1° C. *for* ½ *hour when exposed to high temperatures and low humidities. Further they are able to regain this water and recover sufficiently to hatch.*

It is important to notice that when eggs are heated together, so that they are touching, they will cool one another. If they should be in a heap or attached in a cluster, the outer eggs will presumably lose their water first, and thus keep the eggs inside the cluster cooler longer than if they were lying separately. This almost certainly accounts for the fact that more eggs survived when a larger number were heated together at low humidities than when smaller numbers were heated. It also explains why the loss of weight in small batches was greater than in large batches.

PRACTICAL APPLICATION OF RESULTS

An important point affecting control work which emerges from these experiments is the great resistance of the eggs to heat. The fact that the eggs so easily escape observation in practice makes it difficult to know whether or not they have been killed, and it may easily be that all the adults, larvae and pupae are killed when most of the eggs remain alive.

Table XII gives the times of exposure necessary to kill eggs at eight different temperatures, and Table XIII summarizes various experiments and shows the time required for a complete kill of the various stages.

Table XII

Eggs (normal stock), 1–24 hours old. To show the exposures necessary for a total kill at different temperatures. Giving the percentage hatches of eggs for exposures at and near the fatal point. Humidity 8–10 *per cent*

Time of exposure	Temperature (° C.)							
	43	45	47	49	50	51	53	55
1 min.	—	—	—	—	—	—	—	12·0
2 ,,	—	—	—	—	—	—	55·0	0·0
5 ,,	—	—	—	—	0·6	7·9	0·0	0·0
10 ,,	—	—	—	—	—	0·0	0·0	—
15 ,,	—	—	—	1·0	0·0	0·0	—	—
30 ,,	—	—	—	0·0	0·0	—	—	—
45 ,,	—	—	—	0·0	—	—	—	—
1 hour	—	—	15·0	—	—	—	—	—
2 hours	—	—	0·4	—	—	—	—	—
2¼ ,,	—	—	0·0	—	—	—	—	—
5 ,,	—	28·0	—	—	—	—	—	—
6 ,,	20·6	0·0	—	—	—	—	—	—
7 ,,	—	0·0	—	—	—	—	—	—
12 ,,	0·0	—	—	—	—	—	—	—
15 ,,	0·0	—	—	—	—	—	—	—

Table XIII

Showing the times of exposure needed for a complete kill of the various stages, at 45° C. *and three different humidities. Normal stock*

	1–8 % R.H.	70 % R.H.	95 % R.H.
Eggs, 1 day old	6 hours	4 hours	3 hours
Eggs, 3 days old	1 hour	½ hour	1¼ hours
Newly hatched larvae	25 min.	45 min.	25 min.
Feeding larvae	40 min.	40 min.	35 min.
Last-stage larvae	1¾ hours	2 hours	1¾ hours
Prepupae	1 hour	1¼ hours	1 hour
Pupae	1¾ hours	2¼ hours	2 hours
Adults: Female	43 min.	45 min.	15 min.
Male	23 min.	45 min.	15 min.

In order to show whether *E. kühniella* could be killed under more normal conditions than those of the experiments already described, four cultures of normal stock were taken, undisturbed in their tins, and heated in an incubator. The flour was about 1 in. deep, and the heat coming from all sides penetrated quickly. In the cultures were placed tubes of newly laid eggs, about 250 in each culture. A few minutes after placing in the incubator the temperature at the top of the flour of the cultures was 31° C. After 1 hour it was 39° C., 2 hours 41° C., 6 hours 45·5° C., and at 8 hours 46° C., when they were taken out. These were the lowest temperatures recorded in any of the cultures. In the culture with the highest temperature 50° C. was reached. In none of the four tins were any *E. kühniella* found to be alive afterwards. Another similar culture was heated for just over 2 hours, during which the temperature rose from

45 to 48° C. No insects survived. It is in the favour of the control of this moth that no stage is likely to be very much concealed, for unlike many of the coleopterous pests of flour, the larvae work on the surface of their food and are thus more easily reached by heat.

It should be noticed that a better control of the eggs is obtained when the humidity is high. A high humidity is not favourable to any stage except the adults, and even at a high humidity the adults die before any other stage.

DISCUSSION

It appears that there may be two main causes of death during heating:

(1) *By drying*, so that the water content is reduced below the amount necessary for life. Water will be lost during heating unless the air is quite saturated. This last condition was not attained in any of the foregoing experiments.

If death is due to drying then insects with a large evaporating surface will lose water more quickly than those with a relatively smaller surface. This may partly explain why moths, with their large surface, are killed more quickly than larvae, why larvae are killed more quickly than eggs, and why the larger female pupae and moths are slightly more resistant than males (Table XIII).

(2) *By direct injury to metabolism.* Mellanby (9) suggests that heat kills insects by "some internal reaction, the products of which cannot be removed sufficiently fast at high temperatures". Two poisonous products which may accumulate at high temperatures are carbon dioxide and lactic acid, as the results of increased respiration. Death at high temperatures may be due to the accumulation of these poisons. If this is so then the stages with lower rates of metabolism may be more resistant.

From what is known of the metabolism of other insects, we would expect to find in the *Ephestia egg* small oxygen consumption when first laid, but rapidly increasing up to hatching; in the *feeding larva* higher oxygen consumption than in the egg; in the *pupa* less oxygen consumption than in the larva; and in the *adult* more oxygen consumption than in any of the other stages. This runs closely parallel with the varying resistance of *E. kühniella* to heat (Table XIII). The newly laid egg is the most resistant stage; the feeding larvae are much less resistant; the pupae are more resistant than the feeding larvae, and the adults are the least resistant stage. It will be noticed that the prepupa does not fit into this scheme. In this stage the cause of death is probably different to that in any other stage (see p. 813).

The writer intends to investigate these various points further.

SUMMARY

Experiments are described on the resistance to heat of *E. kühniella* in all its stages.

With *eggs* exposed to 45°C. (113° F.) and 47°C. (116·5° F.) it is seen that:

(1) One-day-old eggs are more resistant than older ones.

(2) Eggs are more resistant to heat at low than at high humidities.

(3) Eggs appear to be able to cool themselves at low humidities in a high temperature by the evaporation of water.

(4) After heating, eggs can regain water they have lost if they are kept in a moist atmosphere.

(5) After heating at low humidities, there is a bigger survival if the eggs are kept in a moist atmosphere than if they are kept in a dry atmosphere.

(6) There is great variation in stock direct from a mill. Eggs from different pairs of moths may have widely different degrees of resistance.

(7) Stock which has been inbred in the insectaries for twelve generations, shows less variation in resistance than stock direct from a mill.

Larvae are much less resistant than eggs. Newly hatched larvae and all larvae until they are full grown have about the same resistance. Last-stage larvae are far more resistant than other larvae.

Pupae are more resistant than feeding larvae.

Adults are the most susceptible stage to heat. At low humidities the females are more resistant than the males.

Experiments on all stages in undisturbed cultures show that a temperature of 45–46°C., maintained for 3 hours, kills all stages.

This work was carried out under the direction of Prof. J. W. Munro, to whom I am grateful for his constant encouragement. I have to thank Mr G. V. B. Herford for his help in devising and constructing the apparatus and reading through the typescript, and I am also indebted to Dr O. W. Richards for his helpful advice on many occasions, and to Mrs Richards who kindly supplied me with a highly inbred strain of *E. kühniella*. Lastly I thank Dr G. Fraenkel for many important suggestions, especially those contained in the discussion.

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STUDIES ON *OSCINELLA FRIT* LINN.

INHERITANCE OF RESISTANCE OF OATS TO ATTACK BY THE FLY AND THE COMBINATION OF RESISTANCE WITH OTHER CHARACTERS OF AGRICULTURAL IMPORTANCE

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(With 1 Text-figure)

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INTRODUCTION

THE fact that differences existed between varieties of oats in extent of attack by the frit-fly was established during the period 1919–27 while the biology of the frit-fly was being studied.[1] Since 1927 attempts have been made to demonstrate inheritance of resistance to attack and, at the same time, to breed strains of oats resistant to attack by the fly, yet possessing characters of agricultural value. This paper presents the results obtained from one or two of a large series of hybridizations.[1]

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[1] Data acquired during this period have been published in a series of papers in *Ann. appl. Biol.* 1921 *et seq.*

There are three obvious ways of attempting to solve the economic problem presented by the frit-fly:

(1) It is possible to move the susceptible period of plant growth out of the fly prevalence period by altering the date of sowing; this may be accomplished by sowing winter oats instead of spring oats, or alternatively by sowing spring oats early in the season, although such simple procedure is not always possible, because

(*a*) winter oats, in general, have not been very resistant to conditions brought about by frost, i.e. they have lacked winter hardiness; recently, however, Dr Hunter's new oat "Resistance" has proved to be of value, inasmuch as it combines a considerable degree of winter hardiness with grain and straw quality;

(*b*) such strains provide only a partial solution of the problem, since if the producer has difficulty in preparing the land in spring, owing to inclement weather, or is committed to the late removal of a crop, he requires an oat which he can sow late in spring with assurance that it will not be devastated by the frit-fly. Yield in the case of the present English types decreases rapidly if the sowing date is later than the middle of March in this area; in such cases, the plant is approximately in the five-leaf stage when the fly begins to appear and may suffer both primary shoot and tiller losses.

(2) It may be possible to select or breed strains able to recover from attack, that is, able to produce panicles of economic value in a shortened growing period; until, however, limitation or prevention of attack has been proved to be impossible of attainment, investigation of tolerance would seem to be less preferable.

(3) Limitation of attack by utilization of the character, resistance, seems to offer a better prospect of solving the problem if inheritance of resistance can be established and suitable parent types discovered.

A statistical analysis of the extents of infestation by the frit-fly of the variety Victory and a strain derived from a cross Victory × Lochows Yellow, showed that Victory × Lochows Yellow apparently inherited the resistance to attack by the fly possessed by Lochows Yellow (1). This observation suggested that it might be possible to breed resistance to attack into the English varieties of oats, the best of which are highly susceptible to attack by this fly; and further, the data suggested that transgressive segregation of the character, resistance, might occur.

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[1] These investigations have been carried out with the aid of grants from the Ministry of Agriculture.

A. TECHNIQUE

The technique employed, although based on Mendelian principles, differs from that in general use amongst plant breeders. It is dependent on the presence of a natural and effective "inoculum" in the field. Well-known varieties, possessing characters other than resistance to attack which make them valuable in cultivation, are crossed with Swedish varieties possessing the valuable character of resistance to attack, but which otherwise are unsuitable for English conditions. No type of oat immune to attack has been found, and the available material exhibits variation in degree of resistance only, the maximum difference between the most susceptible types and the most resistant types at present known being about 30 per cent; also, resistance being only partial, the characters moderate resistance and low resistance are not readily differentiated.

The F_1 generation resulting from a cross between the two types of parents is grown under such spatial conditions as will enable it to produce a large number of seeds per plant (normally 2500–3000). Seeds of the F_2 generation are sown at a spacing of 2×6 in. late in spring (22 April) to throw the four-leaf stage of the plant into the period of maximum fly prevalence (23–30 May), because when many selections are being examined it is essential to plan for maximum elimination each year; consequently extent of attack, given suitable conditions for the fly, such as absence of rain from the time the plants appear above ground, may frequently be abnormally high; this deliberate planning for maximum attack produces an order of loss quite outside that experienced under normal conditions in the field. Therefore differences between, and not actual, rates of infestation, must be considered when estimating possible economic values. On a field scale the primary shoots of the plants provide the nearest approach to a homogeneous population and only such shoots are examined, plants having attacked primaries being discarded; the remaining plants are harvested, threshed and winnowed by hand, being then selected for seed quality, after comparison with standard varieties grown as controls. During the fourth year, the selections undergo further trial to eliminate those which may have accidentally escaped attack by the fly during the previous year; in this case sixty plants of each selection is about the maximum number permitted by both yield and spatial considerations and these are set out in two rows of thirty plants each, standard types being interspersed regularly in the beds. One row is available for individual plant examination, while the other provides material for the following year. During the fifth year each selection set out consists of thirty closely related progenies and the whole block of progenies should, by this time, show evidence of resistance under similar trial. It then remains to select for differences in characters of agricultural value and to make additional trials for 3 years to demonstrate satisfactorily that resistance is held under varied conditions of growth. Single-line selection during the F_8–F_{10} generations is normally required to obtain a reasonably "pure" strain, before multiplication.

The detailed examination of single plants demanded by this method limited the material which could be handled within the 10 days or so during which the primary shoots could be recognized with certainty (identification marking of foliage having failed) to about 50,000 plants; for this reason an investigation committee of the Agricultural Research Council suggested that, from the purely economic point of view, a wider field of choice might be obtained by utilizing mass cultures, basing the method on the fact that heavily infested plants would yield less than those lightly infested. This method, with certain modifications, is now under trial.

Latin square experimentation can only be adopted to a limited extent under cage conditions, as it requires too big an area per strain and more seed per strain than is usually available in the early stages.

B. EXPERIMENTAL DATA

No attempt has been made to breed large numbers of F_1 seed and to expose the F_1 plants to attack, to determine the dominance or otherwise of resistance, because in the field the result would be vitiated by irregularity of attack and in the insectary by abnormal conditions of growth.

(1) *Parents and standards*

The resistant parent varieties used were Spet, a "land" variety from Småland, Sweden; Hede, from Denmark; and Sommar (Summer) from Gotland, in the Baltic. No parent type has yet been found to exceed Summer in resistance, although some seventy varieties from this country, Sweden, Finland, U.S.A. and even Australia, have been examined.[1]

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[1] Row trials showed that none of the following approached the Swedish varieties as resistant parent types, being all either more susceptible than Victory or giving results within 10 per cent of that obtained for Victory: *Avena brevis*, *A. fatua*, *A. nuda*, *A. sterilis*, *A. strigosa*; the varieties Algerian (white and black), Argentine White; from Australia—

Many varieties, such as Victory, Abundance, Star, King, Gris de houdan, Noir trés hâtive, Grey Winter, White Argentine, etc., possessing good agricultural characters, but not resistance to attack, have been used in conjunction with resistant types in making seventy-five or so distinct hybridizations.

That resistance is a definite character, or association of characters, is clearly shown by the data set out in Table I and derived from parent and control varieties for which records are available over the period of years 1927–35. Until 1932, standard errors were estimated for individual varieties; after that year the standard error of the mean difference was calculated from data derived from either random block or Latin square lay-outs.

Table I

Data from parent types, years 1927–35. *Percentage losses of primary shoot, with standard errors*

	Sweden 1927	1928	1929	1930	1931	1932	1933	1934	1935
Victory	31 ±2·0	—	80 ±1·5	42 ±1·2	79 ±1·7	24 ±1·7	66 ±2·0	98	94
King	31 ±3·6	90 ±2·0	75 ±3·1	43 ±3·6	83 ±1·5	29 ±2·1	70 ±1·9	97	95
Star	43 ±3·8	—	73 ±1·8	40 ±2·2	75 ±1·9	26 ±2·5	64 ±1·8	99	96
Spet	5 ±2·0	56 ±1·9	37 ±1·3	24 ±1·7	53 ±2·1	11 ±1·3	29 ±1·9	75	75
Summer	4 ±1·7	—	44 ±2·4	18 ±2·0	49 ±2·0	8 ±1·1	26 ±1·7	59	65
Standard error of mean difference, years 1932–5						±2·1	±2·4	±3·8	±3·0

In all cases the susceptible types, Victory, King, and Star, are significantly more infested than are the resistant types, Spet and Summer. Hede, the third resistant variety, has been omitted latterly owing to its invariable habit of transmitting pronounced tillering capacity, which complicates examination of cultures unnecessarily at the present stage.

(2) *Annual variations in extent of attack, standards and certain selections*

Data collected during the earlier investigations seemed to indicate that the annual variations in extent of infestation were inversely related to temperature variations, but the extended series when plotted assumed an elliptic form, indicating the operation of other factors. Wet weather

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Admiral, Adonis, Advocate, Amery, Baxter, Belar, Beta, Bimbi, Binda, Birdwood, Bombo, Boppy, Buddah, Glen Innes, Gidgee, Guyra, Imbros Island, Kareela, Kelvin, Kelsalla, Kendall, Kiah, Kurri, Lachlan, Laggan, Mulga, Myall, Palestine, Sunrise, Walla, Weston, and Woodford; Banner, Chunking, Cornish Black, Crown, Dansk, Fulghum; from Finland—Esa, Jalostettu, Nopsa, Osmo II, Pelsonkawa, Tuotto and Veikko; Gris de houdan, Italian, Kanota, Leader, Naisgaard, Noir trés hâtive, Potato (Black), Red texas, Tolo, Thousand Dollar, White Horse and Wexford Tawny. Albion, Giant Eliza, Prolific, and Waterloo gave results about the mean of resistant and susceptible standards.

proves to be a restrictive factor to the fly; as the observational period is limited to 8 years the number of wet days occurring during the period of growth from the appearance of the plant above ground to the four-leaf stage (when susceptibility begins to diminish rapidly) was taken as an approximate measure of wetness, with the result that the partial correlation between extent of attack and temperature, after elimination of the number of wet days, was −0·297, while the partial correlation between extent of attack and number of wet days, after elimination of

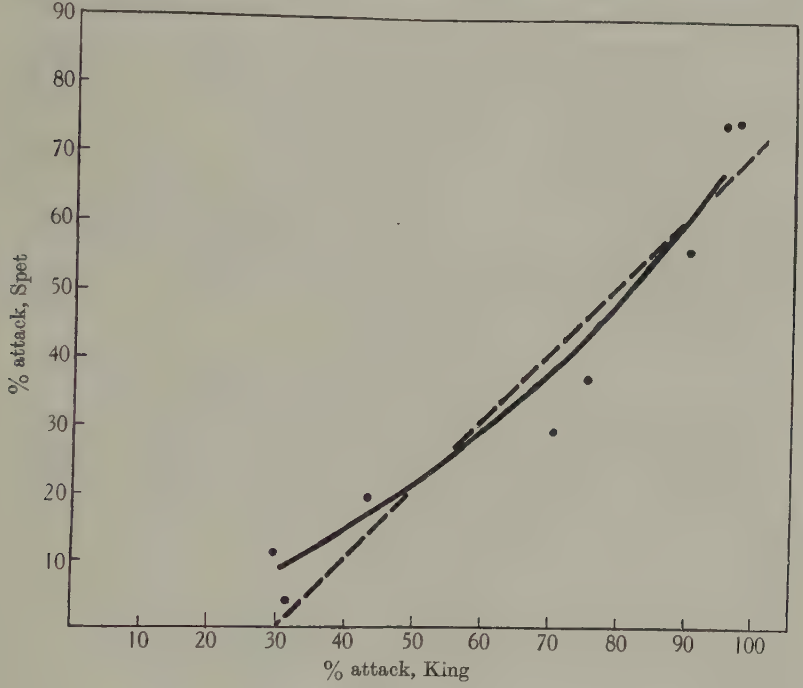

Fig. 1.

Table II

	Spet Resistant	King Susceptible	% difference between varieties	Difference from mean difference
1927	4	31	27	− 1
1932	11	29	18	−10
1930	24	43	19	− 9
1933	29	70	41	+13
1929	37	75	38	+10
1931	53	83	30	+ 2
1928	56	90	34	+ 6
1934	75	95	20	− 8
1935	75	98	23	− 5
		Mean	28	

temperature, was $-0{\cdot}931$. No great accuracy is claimed for the degree of partial correlation, owing to the number of records being so limited, but the indication is of value, as, over a longer period of observation, it promises some explanation of the annual variations.

A further important point is the tendency of the differences to increase in the middle of the range of attack. This is exemplified by the two varieties Spet and King (*vide* Table II and Fig. 1), the latter showing the curve fitted to the data by my colleague Dr Murray.

In Tables III the percentage losses of primary shoots are expressed as differences from means, which show more clearly the effect of differences in resistance. The same table shows seven selections from crosses between Spet × King and Spet × Star which have been carried on for a number of years, there being evidence of inheritance of resistance. The evidence derived from the earlier generations is only to be accepted after confirmation by results of a similar trend with larger populations; Sept × King 3056 and 3055 were discarded for high husk content, 3076 and 3049 for liability to lodge and Spet × Star 3135 for lateness in exserting panicle (8–9 days later than Victory).

Table III

Standards, parents and selected crosses. Percentage losses of primary shoots expressed as differences from means

	1928	1929	1930	1931	1932	1933	1934	1935	Mean
Victory	—	+29	+22	+15	+ 6	+18	+ 9	+11	+16
Star	—	+22	+20	+11	+ 8	+16	+11	+11	+14
King	+34	+24	+23	+19	+11	+22	+10	+10	+19
Spet	+ 0	−14	+ 4	−11	− 7	−19	−10	−12	− 9
Summer	—	− 7	− 2	−15	−10	−22	−20	−28	−15
Spet × King:									
3056	+14	− 3	− 5	− 2	+ 3	0	—	—	—
3055	+14	− 3	−10	− 3	+12	—	—	—	—
3076	− 6	−20	− 7	− 4	− 1	− 3	0	—	—
3049	+11	− 3	− 6	+ 1	− 2	+ 5	+ 3	—	—
Spet × Star:									
3173	−35	− 1	−14	− 5	− 8	−13	− 1	− 2	− 6*
3176	−35	− 1	−10	− 4	− 6	− 5	—	+ 3	− 4*
3135	−17	−22	−10	− 4	− 4	—	—	—	—
Mean	56	51	20	64	18	48	85	87	

* Excluding 1928 result, which unduly favours this selection.

For similar reasons, hundreds of other strains have also been discarded. These particular Swedish strains have grain and straw characteristics of low agricultural value compared with English types and inheritance of these characters has led to the discard of many strains of resistant hybrid material. Selection is based on possession of the following characters in order: (1) inheritance of resistance, i.e. there must be a significant difference between the new strain and the susceptible standard; (2) standing power of the straw, a strain that lodges being useless commercially; (3) colour of seed; (4) capacity for tiller and panicle production; (5) size and character of panicle; (6) 1000-grain weight of seed; (7) percentage of husk in grain; (8) fineness of straw; (9) exsertion of panicle; (10) recovery power.[1]

(3) *Spet × Star Selection* 3173 *compared with standards*

(*a*) *Inheritance of resistance*

The accumulated evidence of the period 1929–33 indicated that the strain Spet × Star 3173 appeared to possess the characters required. The history of the selection is shown in Table IV; strains were discarded for apparent lack of resistance to save space, and also for seed deficiencies. Each of the two strains used during 1932 was taken at random from the corresponding 1931 material.

The data show clearly that, in each year, the selection 3173 was significantly more resistant than the susceptible parent, Star, notwithstanding the irregularity of attack. This irregularity appears to be due to the fact that the fly drifts over the crop and does not make a determined effort to search for susceptible plants.[2]

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[1] Losses by bird damage both in spring and autumn have to be prevented by caging, which does not favour growth. This, together with an abnormal sowing date, makes selection on a quality basis difficult and comparison with standard varieties essential.

[2] This is shown by the results of a "shelter" experiment in which plots of 150 Victory plants, planted 2 × 6 in., were sheltered from invasion by the fly by strips of hessian cloth of varying heights.

"Shelter" experiment

Individual "shelter" plot results, showing height of shelter in feet and corresponding percentage extent of attack on primary shoots

Height in feet							
0	½	1	2	3	4	Mean	S.E. per plot
86·8	62·6	44·3	5·8	0·6	0·5	33·2	36·7%

S.E. of difference between two means of six plots = 7·03, therefore differences of fourteen or more are significant, *z* being 0·7058 for 1 per cent distribution.

Analysis of variance

Due to	Degrees of freedom	Mean square	$\frac{1}{2}\log_e$	z
Rows	5	74·4	2·158	0·342
Columns	5	117·4	2·380	0·120
Treatment	5	8092·8	4·500	2·000
Error	20	148·1	2·500	

The regularity of the decrease in extent of attack indicates a low drift of fly over the whole area. There is, consequently, a tendency for high infestation to occur in areas carrying susceptible types.

Table IV

History of Spet × Star selection 3173, *F_3 generation in* 1928

Year ...	1928	1929	1930	1931	1932	1933	1934	1935	Strain identification
No. of plants examined ...	28	4[1]	30	600	750	4156	900[2]	7200[3] [2]	
Percentage loss of primary shoots	21	50	6	41	—	—	—	—	*a*
				60	14	48	—	93	*b*
				54	7	28	—	—	*c*
				—	13	47	—	89	
				48	13	—	—	—	*d*
				—	14	—	—	—	
				67	—	—	—	—	*e*
				44	13	39	—	—	*f*
				—	24	—	—	—	
				64	23	—	—	—	*g*
				—	4	35	—	90	
				62	9	39	—	—	*h*
				—	14	33	—	89	
				58	0	36	—	—	*i*
				—	29	—	—	—	
				69	—	—	—	—	*j*
				36	0	36	—	88	*k*
				—	0	32	—	—	
				57	10	41	—	87	*l*
				—	6	34	—	—	
				62	4	26	—	88	*m*
				—	4	24	—	—	
				62	8	37	—	—	*n*
				—	7	32	—	—	
				81	—	—	—	—	*o*
				74	—	—	—	—	*p*
				70	—	—	—	—	*q*
				61	5	28	—	—	*r*
				—	0	33	—	—	
				68	—	—	—	—	*s*
				42	12	43	—	—	*t*
				—	8	26	—	90	
				—	—	—	84[4]	*vide* p. 832 for S.E and standard values	
Mean				59 ± 2·6	10 ± 1·5	35 ± 1·5[5]			
Spet[6]	56	37	24	53	11	29	77		
Star	—	73	40	75	26	64	92 ± 1·5		

[1] Remainder of row of thirty plants destroyed by wireworm.

[2] Latin squares.

[3] From material of same strain sown early, 1934.

[4] Sowing from bulk.

[5] Mean attack on twelve other lines, taken from other selections sown early during 1932 = 34 per cent. 1932 and 1933 data, correlation coefficient $r = +0{\cdot}54 \pm 0{\cdot}11$.

[6] For errors of standards, *vide* Table I.

The Latin square trial of the bulked material of the selection 3173 (referred to in Table IV), together with other selections made during 1934, gave the following results which confirm, by accepted experimental methods, the inheritance of resistance.

Standards and certain Spet × Star and Spet × King selections, 1934

Individual varieties and selections, 1934

Victory	Star	Spet	Spet × Star 3173	Spet × King 3076	Spet × King 3049	Mean	S.E. per plot
92·5	91·8	77·2	83·8	85·1	88·0	86·3	3·02 %

S.E. of difference between means of six plots = ±1·49.

Difference of 3·0 or more between results is significant, z being 0·7058 for 1 per cent distribution.

Analysis of variance

Due to	Degrees of freedom	Mean square	$\frac{1}{2}\log_e$	z
Rows	5	86·12	2·225	1·266
Columns	5	27·46	1·657	0·698
Strain	5	190·26	2·622	1·663
Error	20	6·80	0·959	

It is clear therefore that:

(1) Spet is significantly below all others;

(2) Victory and Star do not differ significantly;

(3) the selections are significantly below Victory and Star, 3049 being least resistant; some degree of resistance has in each case been inherited from Spet.

Evidence from the row method of previous years had placed selection 3173 in the first position and selection 3049 last, the other being intermediate; the latter selections, although discarded for reasons mentioned on p. 828, were included in order to obtain evidence of the degree of efficiency of the row method, by means of which a much greater number of selections can be dealt with at one time as compared with the Latin square method.

In an endeavour to obtain some guidance from the mass of experimental evidence available over a period of years for further strain selection, all the available data for the three characters, comparative extent of attack, 1000-grain weight and percentage of husk, were combined and expressed as means, which are indicated on p. 832.

Strains k and b apparently differed markedly from the rest in extent of attack and there were certain differences in the cases of the other characters of obvious value if constant, e.g. low husk of strain h. These strains were therefore included in Latin square experiments made during 1935 together with strains giving average values to determine the degree of variation. In the following sections experimental data derived mainly from the 1935 Latin square experiments are given which show (b) the establishment of a certain degree of "purity" in regard to inheritance of resistance, and that the combination of resistance and other characters of agricultural importance is possible, namely, (*c*) standing power of straw, (*d*) tiller and panicle production, (*e*) size of panicle, (*f*) 1000-grain weight, (*g*) percentage of husk, (*h*) time of exsertion of panicle and (*i*) recovery power after attack. In each case comparison with parent and standard types is made.

Mean differences from means, 3173 *selection*

Strains included in Latin squares	Extent of attack	1000-grain weight	% of husk
k	−9	−2·3	+1·3
t	−3	+1·2	+0·1
m	−2	+0·8	−0·9
	−1	−1·3	+0·2
	−1	−0·8	+1·0
	0	−0·7	+0·7
i	+1	+0·4	−0·4
l	+2	+0·7	−0·6
g	+2	+0·1	−0·3
	+2	−0·1	+0·8
h	+3	0	−1·9
b	+8	+1·4	0

(*b*) "*Purity*" *of strains of selection* 3173.

Selection 3173, *first group of strains,* 1935

Individual strains of 3173

Spet	Star	*k*	*h*	*t*	*b*	Mean	S.E. per plot
88·4	98·3	87·7	88·8	90·1	92·8	91·0	2·9 %

S.E. of difference between two means of six plots = ±1·54.
Difference of 3·1 or more between results is significant, *z* being 0·7058 for 1 per cent distribution.

Analysis of variance

Due to	Degrees of freedom	Mean square	$\frac{1}{2}\log_e$	*z*
Rows	5	18·04	1·446	0·468
Columns	5	23·23	1·572	0·594
Strain	5	96·35	2·282	1·304
Error	20	7·08	0·978	

It is clear that (1) Sept and all the strains are more resistant than Star, and (2) all the strains, except strain *b*, are not significantly different from Spet. Strain *k* gives the lowest figure and strain *b* the highest, according to expectation and their difference is significant, even at this extremely high rate of infestation.

Selection 3173, *second group of strains*, 1935

Individual strains of 3173

Spet	Star	*l*	*m*	*i*	*g*	Mean	S.E. per plot
81·5	96·9	86·8	88·2	88·6	90·2	88·4	3·9 %

S.E. of difference between two means of six plots = ±2·0.

Difference of 4·0 or more between results is significant, *z* being 0·7058 for 1 per cent distribution.

Analysis of variance

Due to	Degrees of freedom	Mean square	$\frac{1}{2}\log_e$	*z*
Rows	5	80·16	2·192	0·912
Columns	5	18·10	1·448	0·168
Strain	5	152·03	2·518	1·238
Error	20	11·93	1·280	

It is clear that (1) Spet and all the strains are more resistant than Star, and (2) all the strains are less resistant than Spet, and (3) the strains do not differ amongst themselves; according to past experience there should not have been much difference between these strains. No explanation can be offered to account for the inconsistency of the Spet results. Taking all the data set out in sections (*a*) and (*b*) into consideration, the inference is that resistance is a heritable character and that, so far as this character is concerned, some degree of "purity" has been established. It now remains to consider the possibility of combining resistance with good quality.

(*c*) *Standing power of straw.*

This Spet × Star selection 3173 was outstanding during 1932, being the only line not lodged by a heavy gale which occurred on 30 June. During the 1933 season there were no untoward events of this kind, but during 1934 both it and related lines stood up well against high winds and heavy rains which flattened many other selections and standards (*vide* section (*j*)).

(*d*) *Tiller and panicle production.*

To obtain some idea of the possible yielding value of these strains, tiller and panicle production were determined on 8–9 July 1935, from Latin square plots sown in the cage during the second week of February.

Tiller estimation, selection 3173 *sampled* 8–9 *July* 1935

Individual strains, tillers per plant

	Victory	*h*	*k*	*l*	*t*	*b*	Mean	S.E. per plot
	11·5	12·2	14·9	14·9	15·9	16·3	14·2	16·0 %
As %	100	106	122	122	130	134		

S.E. of difference between two means of six plots = ±1·06.

Difference of 2·1 or more between means is significant, *z* being 0·7058 for 1 per cent distribution.

Analysis of variance

Due to	Degrees of freedom	Mean square	$\frac{1}{2}\log_e$	z
Rows	5	22·89	1·562	0·732
Columns	5	4·72	0·780	—
Strain	5	24·24	1·595	0·769
Error	20	5·22	0·826	

At this stage therefore all the strains except strain *h* are capable of producing more tillers than Victory, while, with the above exception, none of the strains differ significantly amongst themselves to any marked extent. Strain *h* was nearest to Victory at an earlier date (8 May) when the plots were also sampled, with similar results.

Panicles per plant, selection 3173, 1935

Individual strains, panicles per plant

	Victory	*h*	*l*	*b*	*t*	*k*	Mean	S.E. per plot
	4·2	4·4	5·4	5·8	6·2	6·2	5·4	10·5 %
As %	100	105	129	138	148	148		

S.E. of difference between two means of six plots = ±0·32.

Difference of 0·65 or more between means is significant, z being 0·7058 for 1 per cent distribution.

Analysis of variance

Due to	Degrees of freedom	Mean square	$\frac{1}{2}\log_e$	z
Rows	5	1·72	1·422	0·840
Columns	5	0·28	0·514	—
Strain	5	4·74	1·928	1·346
Error	20	0·32	0·582	

Again, all the strains except strain *h* are significantly above Victory but they differ significantly amongst themselves. Strains *k* and *t* produced most panicles per cent of tillers produced (42 and 39 per cent respectively, against 36 per cent for remainder).

(*e*) *Size of panicle.*

The number of spikelets per panicle was distinctly in favour of the established varieties, as may be seen from the following table based on panicles taken at random from plots sown normally.

	No. of panicles	No. of spikelets per panicle				
		Min.	Max.	Mean		S.E.
Victory	25	32	94	62	±	3·42
Spet × Star 3173	40	27	65	45	±	1·28

The panicles of the strain from Spet × Star were more uniform than those of Victory; the error (3·56) of the difference between the means shows that the difference was significant, this strain having a smaller panicle, at its best only equalling the average Victory panicle. Extra panicle production compensates for this deficiency.

As the panicles carry about three-quarters of the number of spikelets carried by Victory, if they average one-third more in number a bulk sowing should yield equally with Victory, under the conditions of freedom from attack and equivalent 1000-grain weight. This equality actually was established in the Cambridge trial (*vide* p. 838). The extra resistance inherited by selection 3173 should make this selection a better yielder than Victory when subject to attack.

(*f*) 1000-*grain weights.*

Comparative data for the 1000-grain weights of controls, parents and the Spet × Star selection 3173 are shown below followed by data from Latin square plots which included strains of 3173 and Victory. The Spet × Star selection, on the mean of the 3 years, was well placed and an independent trial at Cambridge was confirmatory.

1000-*grain weights*

	1932	1933	1934	Mean	1935 (Cambridge)
		Sown, late April			normal date
Victory	35·0	32·5	30·5	32·7	36·3
Star	36·0	33·9	30·5	33·5	—
King	35·5	36·0	32·3	34·6	—
Spet	36·5	36·6	31·9	35·0	—
Summer	36·4	33·6	32·5	34·1	—
Spet × Star 3173 (bulk)	37·3	34·2	36·2	35·8	39·1

1000-*grain weight, selection* 3173, 1935

Individual strains, 1000-*grain weight*

Victory	*k*	*h*	*l*	*b*	*t*	Mean	S.E. per plot
33·3	37·5	38·8	38·9	38·9	39·1	37·2	4·06 %
Corresponding data from bulk sowings of same strains		36·4	36·7	38·9	38·0		

S.E. of difference between two means of six plots = ±0·71, therefore a difference of 1·5 between individual strains is significant, z being 0·7058 for 1 per cent distribution.

Analysis of variance

Due to	Degrees of freedom	Mean square	$\frac{1}{2}\log_e$	z
Rows	5	3·17	0·576	0·164
Columns	5	5·46	0·848	0·436
Strain	5	30·80	1·713	1·301
Error	20	2·28	0·412	

Thus all the strains were superior to Victory under these conditions, while they barely differed amongst themselves.

(*g*) *Percentage of husk in grain.*

From the same materials, the percentages of husk in the grain were estimated from samples taken at random. The Spet × Star selection again compared well with Victory and the other standards, as indicated by the data given in the following table for the years 1932–5 and the Latin square results of 1935.

Percentage of husk

	1932	1933	1934	Mean	1935 (Cambridge)
		Sown, late April			normal date
Victory	27·6	25·6	29·0	27·4	28·6
Star	27·6	24·6	28·3	26·8	
King	26·4	23·2	28·0	25·8	—
Spet	33·9	32·3	35·8	34·0	—
Summer	28·6	28·4	29·3	28·8	—
Spet × Star 3173 (bulk)	25·3	25·6	27·5	26·2	28·5

Percentage of husk, selection 3173, 1935

Individual strains, percentage of husk

Victory	*k*	*h*		*t*	*b*	Mean	S.E. per plot
23·5	21·4	21·5	21·6	22·1	22·5	22·1	3·11 %
Corresponding data from bulk sowings of same strains		21·5	21·4	21·9	23·1		

S.E. of difference between two means of six plots $= \pm 0{\cdot}395$, therefore a difference of 0·79 between individual strains is significant, z being 0·7058 for 1 per cent distribution.

Analysis of variance

Due to	Degrees of freedom	Mean square	$\frac{1}{2}\log_e$ (comparative)	z
Rows	5	0·89	1·092	0·318
Columns	5	2·71	1·650	0·876
Strain	5	3·92	1·833	1·059
Error	20	0·47	0·774	

Victory is significantly above the strains, while the strains do not differ significantly amongst themselves except for strain *b*, which was also higher in other cultures.

As shown by Hunter (2) time of sowing had no effect on husk percentage for, with this selection, February sowings gave a mean of 25·2 per cent while April sowings of the same year gave 25·1 per cent.

(*h*) *Time of exsertion of panicle.*

Means of blocks

	1932	1933	1934	1935
Victory	77	69	57	76
Star	76	68	54	75
King	79	70	56	79
Spet	77	71	54	71
Summer	69	60	48	63
Spet × Star 3173 (bulk)	73	66	52	—

The Spet × Star selection maintained its earliness of panicle exsertion as compared with the normal types, Summer being an exceptionally early variety.

The various strains of the Spet × Star selection 3173 being set out in Latin square formation during 1935, it was convenient to estimate the relative times of shooting of the different strains.

Exsertion of panicles in days after sowing, individual strains, selection 3173, 1935

Victory	*l*	*h*	*k*	*t*	*b*	Mean	S.E. per plot
128	126	124	122	122	121	124	0·9 %

S.E. of difference between two means of six plots = ±0·2.
Difference of 0·4 or more between means is significant, *z* being 1·800 (0·7058 for 1 per cent distribution).

Thus all these strains are earlier than Victory and significant differences in degree of earliness exist between them.

(*i*) *Recovery power.*

As the extent of attack in the late sown Latin square experiments of 1935 was so intense, it was only necessary to remove comparatively few unattacked plants during the examination of the plots and the opportunity arose of studying the recovery power of the attacked plants. It not being possible to support so many scattered panicles of varying length, in the time available, the number of spikelets produced on each plot was estimated by counting the number in a weighed random sample of the weighed total, taken at the end of July. Such a figure takes account of both the number of panicles produced and the number of spikelets per panicle.

Recovery, parent varieties and Spet × Star selection, 3173, *first Latin square*

Individual strains, no. of spikelets per attacked plant

Spet	Star	*b*	*k*	*h*	*t*	Mean	S.E. per plot
51·3	16·2	16·1	22·3	25·9	26·2	26·3	13·4 %

S.E. of difference between two means of six plots = ±2·2.
Difference of 4·4 or more between results is significant, *z* being 0·7058 for 1 per cent distribution.

Analysis of variance

Due to	Degrees of freedom	Mean square	$\frac{1}{2}\log_e$	z
Rows	5	141·7	2·473	1·211
Columns	5	73·0	2·142	0·880
Strain	5	1016·9	3·455	2·193
Error	20	12·5	1·262	

Recovery, parent varieties and Spet × Star selection, 3173, *second Latin square*

Individual strains, no. of spikelets per attacked plant

Spet	Star	*i*	*g*	*m*	*l*	Mean	S.E. per plot
46·9	20·1	22·3	22·6	23·4	23·6	26·4	11·5%

S.E. of difference between two means of six plots = ±1·7.

Difference of 3·4 or more between results is significant, z being 0·7058 for 1 per cent distribution.

Analysis of variance

Due to	Degrees of freedom	Mean square	$\frac{1}{2}\log_e$	z
Rows	5	25·4	1·618	0·508
Columns	5	11·1	1·203	0·093
Strain	5	609·1	3·208	2·098
Error	20	9·2	1·110	

Significant differences occurred in the first square, but not in the second except in the case of Spet. The superiority of Spet is doubtless due to the fact that a smaller number of plants was killed outright and that the tillers were probably more resistant, rather than to any large discrepancy in production per panicle. Actually in the first Latin square, the numbers of spikelets per panicle for the series Spet, Star and strains *b*, *k*, *h* and *t* were 13·3, 12·1, 8·5, 8·9, 11·6 and 10·7 respectively.

Taking all the evidence into consideration, strain *t*, of this Spet × Star selection 3173, may be placed first in a general order of merit; of the eight strains examined in detail it shows the best combination of resistance and other characters of agricultural importance.

(*j*) *Selections* 3173 *and* 3176 *compared with Victory at Cambridge, Latin square field trial*, 1935.

Dr Hunter courteously included these two closely related selections (bulked material) in one of his spring oat trials and extracted the yield data, which has been subjected to statistical analysis as follows:

	Relative mean yield per plot in g.
Victory	929 ± 88
3173 (bulk)	991 ± 91
3176 (bulk)	844 ± 75

None of the differences is significant, the errors being so large, therefore selection 3173 may be considered to be at least as good a yielder as Victory under normal conditions of sowing, so far as the evidence of this independent experiment is concerned.

The material was sampled for both 1000-grain weight and percentage of husk, with the following results:

Strain	1000-grain weight	Percentage of husk
Victory	36·3 ± 0·43	28·6 ± 0·37
3173 (bulk)	39·1 ± 0·37	28·5 ± 0·33
3176 (bulk)	39·9 ± 0·27	30·3 ± 0·32

Both strains were therefore from 7 to 10 per cent better than Victory with regard to 1000-grain weight, and did not differ significantly between themselves; while, with regard to percentage of husk, 3173 was as low as Victory while 3176 was significantly higher.

Dr Hunter offered the following remarks, with permission to include them in this publication:

"No. 3173 comes within easy striking distance of Victory and that under severe conditions of growth. It may do even better in a more favourable oat season. As to straw, both your varieties produced straw of average length and both stood well. Their outstanding characteristic was a pronounced resiliency which was in marked contrast to Victory (a good straw but not characterized by resiliency). Indeed, the one feature that did strike our crop observer in respect of your oats was the feature I mention, associated with comparative fineness. We did not ascertain the yield of straw, but judging by appearance Victory would outyield both.

"I have a note in my book that your varieties were both semi-erect in early growth and that whilst Victory produced erect spear-like leaves, those of these strains were more pendulous. As the erectness of the leaf is almost invariably an index of stiff straw I think it follows that resiliency is a distinct character."

(4) *A Summer × Star selection*

A hybridization between Summer and Star has also provided confirmatory evidence of inheritance of resistance, and the possibility of its combination with good agricultural characters.

The cross was made during 1927, four F_1 generations were grown on during 1928, the F_2 generations being late sown and naturally selected by the fly during 1929. Selection of both white and black strains was continued during the succeeding years, the strains now being in the F_8 generation, and reduced in number to nine.

Table V

Showing numbers of plants examined and percentage losses of primary shoots of parent types and strains from Summer × Star selections

Year	1930	1931	1932	1933	1934	1935
No. of plants examined ...	120	180	210	270	963	4500[1]
Strain *a*[2]	6	61	10	38	77	—
b	—	50	0	46	81	83
c	—	—	—	38	74	—
d	—	—	—	42	80	86
e	8	44	19	48	—	—
f	—	35	0	43	72	77
g	15	44	7	29	67	—
h	—	—	—	29	63	77
i	—	—	6	33	58	79
j	11	25	4	30	55	—
Summer[3]	18	49	8	26	59	68
Star	40	75	26	64	99	96

[1] Latin square.

[2] Different F_3 selections gave rise to strains *a–d*, *e–i* and *j* respectively.

[3] For errors of standards, *vide* Table I.

This Summer × Star material, having given a high correlation for the years 1933 and 1934, $r = +0{\cdot}838 \pm 0{\cdot}066$, a somewhat remarkable figure under the circumstances, was tested for variability of resistance by a Latin square experiment, five of the strains being tried against Summer.

Summer × Star selection, 1935

Individual strains

b	*d*	*f*	*h*	*i*	Summer	Mean	S.E. per plot
83·1	86·3	76·7	77·4	78·7	67·7	78·3	6·9 %

Thus the S.E. of the difference between two means of six plots is 3·14, and a difference of 6·3 or more between the results is significant, z being 1·055, when 0·7058 only is required for 1 per cent distribution.

Analysis of variance

Due to	Degrees of freedom	Mean square	$\frac{1}{2}\log_e$	z
Rows	5	43·35	1·882	0·190
Columns	5	38·99	1·832	0·140
Strain	5	244·40	2·747	1·055
Error	20	29·47	1·692	

It is clear (*a*) that Summer at 67·7 is significantly below all the strains; (*b*) that between strains a difference in resistance exists, strain *b* at 83·1 and strain *d* at 86·3 being significantly higher than others, as was expected from the previous 5 years' results.

The results of this Latin square experiment bear out the evidence of the mean differences from the annual means of extent of attack throughout 5 years, which are as follows:

Strain *b*	+3·0		Strain *h*	−2·5
,, *d*	+2·7		,, *i*	−2·5
,, *f*	−2·5			

The evidence of consistency is valuable.

The 1000-grain weights and husk, percentages were estimated in the case of the 1933 material, with the following results:

	Min.	Mean	Max.
Summer × Star:			
1000-grain weight	32·3	35·2	38·6
% husk	21·8	23·6	26·2
Star:			
1000-grain weight	31·3	33·9	35·9
% husk	24·2	24·6	25·4
Summer:			
1000-grain weight	33·0	33·6	34·2
% husk	27·0	28·4	30·0

Thus, taking the three characters, resistance, grain weight and percentage of husk, into consideration, these strains show considerable promise.

(5) *Spet crosses at Harpenden*

It is of interest to note that Mr Fryer has, since 1932, carried on at Harpenden two Oxford strains, from Summer × Star and Spet × King, together with the standards, this year subjecting them to analysis for frit attack with the following result, which, by his courtesy, I am permitted to include herewith:

Strain	% loss of primary shoot	E^2
Abundance	65	12·3
Hede	43	75·3
Spet	34	5·5
Summer	47	30·3
Summer × Star	54	14·8
Spet × King	42	67·2

Thus Summer × Star and Abundance (susceptible type) alone differ significantly from Spet, while Summer × Star alone fails to differ significantly from Abundance.

The Summer × Star strain was discarded a year or two ago; the Spet × King strain has, however, retained its resistance for 3 years when grown in another locality. The varieties Summer and Spet have apparently reversed their positions as compared with Oxford results, but actually the measure of significance of the difference only equals 2·17 (i.e. chances approximately 22 to 1).

(6) *Possible factors determining varietal differences in resistance*

The fact that there is a certain constancy in the difference between varieties in extent of attack by the fly, throughout the range of attack, seems to imply that such differences are due to characters which vary more or less regularly with variations of environmental conditions.

From one of the Latin square experiments laid out during 1935, plants of Victory and strain *l* of the resistant Spet × Star selection 3173 in the five- to six-leaf stage were broken up into primary shoots and tillers, and the dry matter and the percentages of ash, nitrogen, total and soluble carbohydrate, crude fibre and combined silica in the dry matter determined for the primary shoots, the data obtained being shown in Table VI. The Spet × Star resistant strain *l* therefore produced 28 per cent more dry matter per primary shoot and proved to contain 6·3 per cent more total carbohydrate, 5·2 per cent more soluble carbohydrate and 9·8 per cent more crude fibre than did Victory; each of these differences is significant, but that for percentage of combined silica is not.

Table VI

Strain	Dry matter per plant	% total carbohydrate	% soluble carbohydrate	% crude fibre	% combined silica
3173, *l*, primaries	0·411 ± 0·017	71·63 ± 0·613	52·13 ± 0·479	19·50 ± 0·372	15·36 ± 0·516
Victory, primaries	0·320 ± 0·009	67·37 ± 0·895	49·60 ± 0·921	17·78 ± 0·317	13·80 ± 0·696
Difference, *D*	0·091	4·26	2·53	1·72	1·56
Significance, *D*/*E* (1 % point, 2·57)	4·68	3·92	2·42	3·52	1·80

Any difference between the carbohydrate balances in two strains may be of material importance; a larger percentage of total carbohydrate in the dry matter is associated, in the resistant strain Spet × Star 3173 *l*, with a larger percentage of soluble carbohydrate. A larger percentage of crude fibre, possibly resulting therefrom, may mean either that the resistant strain possesses more smaller cells, or thicker cell walls or more fibrous tissue than does Victory. Any such character would tend to increase the difficulty of larval penetration and increase resistance to attack. An attempt to demonstrate such differences by measurement of stem sections has not been successful, owing partly to the difficulty of making good sections of this material and partly to the degree of variation shown by the cells.

The difference in content of combined silica, while not significant,[1] is suggestive in view of the order of the strains. It is well known that certain wheat varieties resistant to the attack of the Hessian fly contain more silica than do susceptible types.

In this connexion it is of interest to record that the resistant strain *l* contained more total carbohydrate per unit content of nitrogen than did Victory.[2]

Strain	Total carbohydrate/nitrogen mean ratio with standard error
3173, *l*, primaries	29·0 ±1·12
,, tillers	26·5 ±0·93
Victory primaries	22·9 ±1·34
,, tillers	22·1 ±0·95

The difference of 6·1 between the ratios for the primary shoots, has significance (D/E) of the order of 3·48 and the difference of 4·4 for the tillers, a significance of 3·35 (1 per cent point = 2·57). Although this difference between the strains is marked, at the moment it is not clear how it is related to the difference in crude fibre production, etc.

SUMMARY

As the result of studies on *Oscinella frit* during the years previous to 1928, it was considered that an attempt might be made to solve the economic problem presented by this fly by hybridization of oats, using a resistant variety as one parent.

The technique adopted is described and the experimental data show that resistance to attack is an inheritable character (or complex of characters), although difficult of precise measurement. Further, it is shown that agricultural quality and resistance to attack are not incompatible.

Wet weather conditions during the flight of the fly have a very marked effect in limiting the extent of damage to the crop.

It is suggested that varietal differences in extent of attack may be due to varietal differences in crude fibre production or deposition of silica, both tending to increase the larval difficulties and therefore the resistance of the plant.

I have great pleasure in acknowledging the readiness with which Dr Hunter of the School of Agriculture, Cambridge, has given me the

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[1] Only a few samples of ash were available for this determination.

[2] Determined in collaboration with my colleague C. G. T. Morison.

benefit of his experience; also, the valuable criticism which Mr J. C. F. Fryer, of the Ministry of Agriculture Pathological Laboratory, Harpenden, has always placed freely at my disposal.

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SHEEP BLOW-FLY INVESTIGATIONS

III. OBSERVATIONS ON THE CHEMOTROPISM OF *LUCILIA SERICATA* MG.

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In a previous paper of this series (2) it was shown that various putrefying materials attract *Lucilia sericata* Mg., the sheep maggot-fly, to oviposit on live sheep. Since these materials were only effective when in contact with sheep, a combination of two factors is clearly essential for oviposition: (i) supplied by live sheep, probably some volatile sweat constituent, (ii) products of putrefaction. For convenience, these will be referred to as the *S* and *P* factors respectively. A peculiar feature of the *S* factor was its apparent absence from the skin and fleece of a recently slaughtered sheep. A preliminary examination of the chemical nature of the *P* factor showed that ammonia was attractive when placed on live sheep. The present paper describes further experiments on the attractiveness of various chemicals when placed on sheep. It also includes some preliminary tests on the efficiency of repellents in preventing oviposition on sheep treated with an attractive substance.

METHODS

The experiments were carried out in a small paddock behind the laboratory. 5–10 ml. of the test solution were poured on to a piece of cotton wool tied to the fleece of a sheep and after 1–2 hours the treated spot was examined for the presence of eggs. A positive control was run with a substance known to be attractive, usually larval excreta. Most of the experiments were carried out during "heat waves" in July and August when the temperature was over 70° F. Under these conditions, as many as 5–10 clusters of eggs were found with attractive substances and negative results proved reliable if duplicated. In order to measure the degree of attractiveness of different substances, a solution was diluted 2, 4, 8 times, etc., until oviposition no longer resulted. This method generally produced a sharp disappearance of attractiveness; occasionally a dilution was found which gave negative results on one occasion and a *small* number of eggs on another.

VARIATION IN *S* FACTOR

The *S* factor of the same individual sheep appeared to be equally effective throughout these experiments; however, a marked variation was found in different sheep when treated with the same attractant. This is illustrated by the results obtained with solutions of ammonium carbonate when placed on four Welsh sheep (Table I). It will be seen that the minimum attractive dose varied from 0·5 to 4 per cent. Presumably, this indicates that the amount of the *S* factor varies in different sheep. In the 1934 investigations (2), it was noticed that the mature rams attracted flies more readily than lambs and also emitted a stronger "sheep odour". To the human perception, the smell of No. 2, the most attractive to the flies, was less than that of the ram (No. 1) and similar to that of the other yearlings. The *S* factor is, therefore, not an odour which is noticeable to man; however, since it varies from sheep to sheep and, in conjunction with a source of the *P* factor, attracts flies from a distance, it is presumably a volatile substance attracting by its odour.[1]

Table I

Variation in attractiveness of different sheep

	Oviposition				
Ammonium carbonate % ...	4	2	1	0·5	0·25
No. 1, Mature ram	+	+	+	−	−
2, Yearling wether	+	+	+	+	−
3, ,,	+	+	−	−	−
4, ,,	+	−	−	−	−

+ = eggs laid; − = no eggs laid.

TESTS WITH REPELLENTS

The discovery that certain substances attract *L. sericata* to oviposit on sheep should provide a simple method of testing repellents. For accurate investigation of repellent action a standard attractant is required and to this different substances can be added to test their efficiency in preventing oviposition. It will be shown later that certain pure compounds attract *L. sericata* to oviposit on sheep; it is, therefore, now possible to prepare standardized attractants. These were not, however, available at the beginning of the season, and a preliminary series of experiments was carried out with an attractant of biological origin—excreta of *L. sericata* larvae. The same preparation was used throughout;

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[1] My colleague, Dr W. M. Davies, observed that *L. sericata* approach sheep in a *short* time and *upwind*, when a solution of indole and ammonium carbonate, itself unattractive, is placed in a glass container on the back of a sheep.

5 ml., together with various amounts of added substances, were tested by the general method already described, each experiment being repeated at least twice. Duplicate tests rarely showed disagreement; when this did occur, it presumably indicated that the particular concentration had doubtful repellent properties.

Table II

Effect of various substances in preventing oviposition by L. sericata *on sheep*

% added to attractant ...	1·0	0·1
Aniseed oil	±	+
Harness oil	±	+
Mustard oil	−	+
Pine tar oil	−	−
Quinoline	·	+
Naphthalene	+	·
p-Dichlorbenzene	·	+
Camphor	−	+
Phenol	−	+
Nitrobenzene	−	·

− = no eggs laid; + = eggs laid; ± = oviposition found on some occasions, not on others.

It will be seen from Table II that only one of the substances tested—pine tar oil—was strongly repellent, 0·1 per cent of this oil preventing oviposition. Mustard oil, camphor and phenol were repellent when 1 per cent was added to the attractant, but not with 0·1 per cent. It was surprising to find oviposition in the presence of strong-smelling materials such as aniseed oil and quinoline; it was noticed, also, that eggs were laid within 1 cm. of crystals of naphthalene. These results show that *L. sericata* is extremely persistent and cannot readily be prevented from ovipositing on sheep. The method is clearly a critical one and should prove valuable in eliminating useless repellents and suggesting materials worthy of further study. When the weather is hot and sunny, three experiments can be carried out with each sheep in 1 day, and definite results are obtained. This overcomes many practical difficulties which hitherto have prevented a satisfactory study of repellents in the field. It is hoped to carry out a more extensive series of tests next summer with a standard chemical attractant.

NATURE OF *P* FACTOR

Ammonium hydroxide and putrefying materials have been shown to attract *L. sericata* to oviposit on sheep (2). In the present experiments ammonium carbonate proved far more attractive than ammonium hydroxide. Thus, a $0{\cdot}2N$ solution of the carbonate induced oviposition on sheep No. 1, whereas a $0{\cdot}8N$ solution of the hydroxide was not attractive. However, the effect of natural putrefying materials is not entirely due to ammonium carbonate. For example, while the pure compound was unattractive at less than 1 per cent ($0{\cdot}2N$), a sample of larval excreta was attractive at a dilution of 1 in 20, which was found (by estimating the ammonia) to contain only 0·05 per cent of ammonium carbonate. Fractionation of larval excreta did not provide much information as to the nature of any attractants other than ammonium carbonate. The effective compounds are volatile since evaporation to dryness destroyed the attractive properties of this material, but steam distillates did not prove highly attractive. Probably the chemotropic effect of the excreta is due to a mixture of moderately volatile compounds which only distil slowly with steam. Basic compounds are not entirely responsible since much of the attraction disappeared when the substance was made acid, evaporated nearly to dryness and then made alkaline again with sodium carbonate.

Since it is known that various aliphatic alcohols, acids and esters attract houseflies, tests were carried out with compounds of this nature and with certain putrefactive bases. As it seemed possible that ammonium carbonate might be an essential component of the *P* factor, in most of the experiments the test substance was added to a solution of ammonium carbonate, which by itself was not effective (half the minimum attractive concentration). The results are shown in Table III.

Table III

Attraction of various compounds for L. sericata

Series I (tested alone)		Series II (added to $(NH_4)_2CO_3$ solution, ½ minimum attractive concentration)	
Substance	Oviposition on sheep	Substance	Oviposition on sheep
$0{\cdot}1N$ ammonium valerianate	–	$0{\cdot}02N$ acetic acid	–
$0{\cdot}1N$ ammonium butyrate	–	$0{\cdot}02N$ butyric acid	–
$0{\cdot}1N$ ammonium lactate	–	$0{\cdot}02N$ valerianic acid	±
$0{\cdot}2N$ valerianic acid	–	2·0 % ethyl alcohol	–
$0{\cdot}2N$ butyric acid	–	0·1 % amyl alcohol	–
$0{\cdot}2N$ lactic acid	–	0·1 % butyl alcohol	±
100 % butyl acetate	–	$0{\cdot}02N$ ethyl butyrate	±
100 % ethyl butyrate	–	0·05 % sodium sulphide	–
100 % amyl alcohol	–	0·02 % trimethylamine	–
100 % butyl alcohol	–	0·02 % methylamine	–
2 % ethyl alcohol	–	0·01 % indole	+
1 % methylamine	–	0·1 % tyramine hydrochloride	–
		0·1 % histamine phosphate	–

Tests carried out in duplicate: + = large numbers of eggs; – = no eggs; ± = small number of eggs in one test, none in other.

None of the aliphatic compounds proved highly attractive; methylamine, trimethylamine, histamine and tyramine gave negative results. In the previous year's experiments, trimethylamine, ethylamine and an aqueous solution of indole prepared by shaking up crystals with water proved unattractive. An entirely different result was, however, obtained this year with a solution prepared by dissolving indole in a small amount of alcohol and diluting with water. This solution proved strongly attractive and produced oviposition on sheep No. 2 at a concentration of indole as low as 0·005 per cent. Table IV shows the results obtained with indole and the related compounds, skatole (methyl indole) and pyrrole. Both indole and skatole are products of bacterial putrefaction and possess an obnoxious faecal odour.

Table IV

Results obtained with indole, skatole and pyrrole

		Oviposition on sheep	
	%	No. 1	No. 2
Indole	0·01	+	+
	0·005	–	+
	0·0025	–	±
	0·0012	.	–
Skatole	0·01	–	+
	0·005	–	–
Pyrrole	0·01	–	–

± indicates doubtful response.

It will be seen from the table that skatole was also highly attractive, but less so than indole; pyrrole had no effect at a concentration of 0·01 per cent. The attractant properties of mixtures of ammonium carbonate and indole appeared to be equal to, or slightly greater than the sum of those of the components. Indole and skatole had no attraction for blow-flies when not placed on sheep, either in the field or in cages; Howlett (3) observed in India that *Sarcophaga* sp. oviposited in response to a solution of skatole.

Discussion

The attractiveness of sheep for *L. sericata* varies considerably among individuals (Table I), and this may represent an important new factor in the susceptibility of sheep to blow-fly attack. A sheep (such as No. 2), which attracts flies when treated with low concentrations of ammonium carbonate or indole, would be more liable: (*a*) to become blown; (*b*) to be reblown if the wound after treatment did not heal cleanly. The relative attractiveness of sheep can now be readily measured by means of solutions of ammonium carbonate or indole. This subject merits further investigation, and it is hoped to examine next season the effect of age, sex, wool type, etc., on attractiveness. The discovery is important in relation to experimental research on the sheep maggot problem, for, in tests on preventive measures, especially repellents, and on dressings for strikes, the selection of sheep of equal attractiveness should increase the probability of obtaining significant results.

The stimulus which induces *L. sericata* to oviposit on sheep is supplied by a combination of two factors, provided by the sheep itself (*S* factor) and by products of putrefaction (*P* factor) (2). The present experiments have shown that ammonium carbonate, indole or skatole will supply the *P* factor, as solutions of these compounds induce oviposition on sheep. These are probably not the only attractive substances present in putrefying materials; nevertheless, as a result of these observations, it is now possible to prepare standardized attractant solutions which should prove valuable for testing repellents and measuring the relative attractiveness of different sheep.

Although several workers have studied the chemotropic responses of the house-fly, *Musca domestica*, our knowledge of the chemotropism of blow-flies is extremely meagre. It is of interest that the responses of *Lucilia sericata* resemble in some respects those of *Musca domestica*. The most peculiar feature of the chemotropism of the sheep blow-fly is the dual nature of the stimulus which attracts females to oviposit on sheep. The house-fly, like the sheep blowfly, oviposits in response to ammonium carbonate, but only when an additional factor is supplied. Crumb & Lyon (1) and the Richardsons (5) observed that a mixture of bran and ammonium carbonate attracts house-flies to oviposit; Richardson (4) also found oviposition with a mixture of ammonium carbonate and butyric acid, neither substance alone having any effect. These results show that, in chemotropic experiments, it is important to test mixtures of various substances, as trials with individual compounds may give misleading results.

SUMMARY

1. The attraction for *Lucilia sericata* Mg. of putrefying substances, when placed on sheep, seems to be due largely to the presence of indole, skatole and ammonium carbonate. When dilute solutions of these compounds are placed in the fleece of live sheep, *L. sericata* females are

attracted to oviposit. This represents an important advance since standardized attractant solutions are now readily available.

2. A technique for testing repellents has been devised which is based on this tropism. The method appears to be critical and is rapid, each test requiring only 2 hours; it should be especially valuable for selecting likely repellents for further investigation.

3. Sheep vary considerably in their attractiveness for *L. sericata*; this may be an important new factor in the susceptibility of sheep to blow-fly attack. The attractiveness of individual sheep can readily be measured with solutions of indole and ammonium carbonate.

I am greatly indebted to the Agricultural Research Council for a grant which has entirely financed this work. I wish to express my appreciation to Dr W. M. Davies for his helpful advice and active interest in this work.

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SHEEP BLOW-FLY INVESTIGATIONS

IV. ON THE CHEMISTRY OF THE FLEECE WITH REFERENCE TO THE SUSCEPTIBILITY OF SHEEP TO BLOW-FLY ATTACK

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Introduction

Holdaway & Mulhearn (3) have recently suggested that the sweat[1] content of the fleece provides an index of the susceptibility of sheep to blow-fly strike. In order to test this hypothesis, a series of wool analyses has been carried out, determinations being made of *p*H value, water-soluble matter and grease. In this country (2) the rump and shoulder are the most common sites of attack. Soiling with urine and faeces explains the susceptibility of the rump, but it is difficult to understand why the shoulder region should be liable to attack. If Holdaway & Mulhearn's theory is correct, the suint[1] content of the wool should be higher on the shoulder than on the rest of the back which is less susceptible. An investigation was therefore made of the distribution of suint over the body; analyses were also carried out on wool samples collected from sheep struck during wet weather in the autumn and from some which had not been attacked.

Experimental

Small samples of wool were used weighing about 2 g. *p*H determinations were carried out as soon as possible, usually within 24 hours. Before suint and grease were estimated, the samples were exposed for 4 days to air of 50 per cent relative humidity. All the percentages given are based on the weight of wool after this treatment.

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[1] The word "suint" will be used to designate the water-soluble matter in wool; this is usually regarded as the dry residue from the evaporation of the product of the sudoriferous glands. It will be shown later that Holdaway and Mulhearn's argument depends partly on suint analyses made by other workers; they use the word "sweat" to indicate "dried sweat" (=suint).

Analytical methods

pH was determined colorimetrically with a B.D.H. comparator. A water extract was prepared by shaking the wool with distilled water for a few minutes. 50 ml. proved the most suitable volume for extracting 1 g. of wool; the solution was too cloudy with a smaller quantity of water and the pH was affected by the volume of solvent if this exceeded 70 ml. per g. The grease content was determined by drying the wool and extracting in a Soxhlet extractor with ether, the grease removed being dried and weighed. The suint content was estimated as follows: the wool was placed in a flask, treated with 50 times its weight of water and left to stand overnight; the solution was then poured into a beaker, acidified with one drop of strong hydrochloric acid and repeatedly filtered until the filtrate became clear; 50 ml. of this solution were evaporated in a tared basin which was dried at 100° C. and weighed. In the later samples, on which grease determinations were carried out, the water-soluble matter was estimated in the residue after extraction with ether.

Table I

Diagrammatic representation of fleece showing pH *and suint content of basal wool in different regions. Samples collected from ewe on* 21 *June* 1935

	C	B	A	B	C	
0			17·8 (8·8) 17·4 (8·9) ...			Breast (lower neck)
1		⊗	* 3·0 (7·0)	⊗		
		5·8	3·7——4·8			
2		5·0 (8·2)	4·3 (7·4)	6·2 (8·2)	8·3 (8·3)	Flank
			3·5 (7·4)			
3	7·6 (7·5)		2·2 (7·5)	8·0 (9·2)	8·7 (7·7)	
			1·7			
4		⊗	2·3 (8·4) *	9·3 (8·6) ⊗	7·6 (7·5)	
			Rump	Leg		

* Wool in this region stained yellow. Bracketed figures = pH values. Unbracketed figures = suint percentage. Site of sample to right of suint figure and to left of pH value.

Results with ewes.

In order to obtain a general idea of the distribution of suint, a large number of wool samples were collected from one ewe; most of these samples were taken from the wool close to the skin (approximately the bottom 2 in. of the fleece, referred to as basal wool). Table I shows the values found for the pH and suint content of the basal wool in different parts of the fleece, Table II gives some corresponding figures for the grease content, and Table III shows the distribution of pH, grease and suint along the fibre.

Table II

Grease content of basal wool from different parts of the body. Samples collected from ewe on 21 *June* 1935

Site of sample		Grease (%)
4 A.	Back (hind)	7·1
2·5 A.	Back (mid)	15·1
1 A.	Shoulder	14·8
3 C.	Flank	15·6
0.	Breast	11·0

It will be seen from Table I that along the backbone (A, 1–4) the suint content is low and the reaction approximately neutral; the fluctuations are comparatively small and appear to occur in a random way. On the side of the back (B) and on the flank (C) the suint content is higher; on the breast (0), the wool contains considerably more suint and the reaction is more alkaline. The shoulder region (1 A) does not show marked differences as compared with other parts of the back. These findings agree with the results obtained with other sheep which will be given later.

Table III

Distribution of suint, grease and pH *along the fibre. Samples collected from ewe on* 21 *June* 1935

			Fraction		
Site of sample		...	Distal	Middle	Basal
1 A.	Shoulder:	pH	6·9	7·7	7·4
		Suint %	4·5	6·3	4·0
		Grease %	14·5	13·2	8·6
2·5 A.	Mid-back:	pH	5·8	7·0	7·4
		Suint %	1·5	2·4	3·5
		Grease %	18·6	17·6	15·1
3 C.	Flank:	pH	7·3	8·2	8·4
		Suint %	6·7	8·1	8·0
0.	Breast:	pH	7·9	8·3	9·6
		Suint %	9·7	13·8	21·1
		Grease %	11·4	12·4	10·0

Table III shows that the pH and suint content decrease from the base to the tip of the fibre, whereas the grease content tends to increase; this was found in almost every case, an exception being the discoloured wool from the shoulder. In this latter sample, the pH and suint content were highest in the middle fraction, which was also the most highly coloured.

Suint and pH determinations were also carried out on a further batch of forty-two samples collected from seven Welsh ewes. The results are shown in full in Table IV and in summarized form in Table V.

Table IV

pH and suint content of basal wool from different parts of fleece. Samples collected from seven ewes on 4 June 1935

		pH value						
Sheep no. ... Region (see Table I):		1	2	3	4	5	6	7
1 A.	Shoulder	6·7	7·2	7·3	7·3	7·5	7·3	7·4
1·5 B.	Side of shoulder	7·1	7·6	7·3	7·1	7·4	8·3	7·8
2·5 A.	Mid-back	6·8	7·2	8·0	7·0	7·4	6·7	7·5
4 A.	Hind part of back	6·7	7·5	6·4	6·9	7·1	7·2	6·9
2·5 C.	Flank	7·7	8·5	7·5	7·7	7·7	8·6	8·7
0.	Breast	9·2	8·9	8·5	9·2	9·1	9·2	9·2

		Suint %						
Sheep no. ... Region (see Table I):		1	2	3	4	5	6	7
1 A.	Shoulder	4·9	4·8	8·9	5·3	7·5	8·0	9·7
1·5 B.	Side of shoulder	7·7	7·7	8·9	8·0	10·5	10·2	9·8
2·5 A.	Mid-back	5·9	5·8	12·1	6·0	10·4	5·6	13·2
4 A.	Hind part of back	3·3	3·5	7·1	7·6	9·6	4·2	9·2
2·5 C.	Flank	8·8	13·7	9·8	16·6	17·4	13·4	16·1
0.	Breast	17·8	17·8	24·1	25·6	23·1	25·3	18·9

Table V

Mean values for different regions calculated from figures in Table IV

	pH		Suint %	
Region	Mean	S.E. ±	Mean	S.E. ±
Shoulder	7·2	0·10	7·1	0·71
Side of shoulder	7·5	0·17	9·0	0·46
Mid-back	7·2	0·17	8·4	1·3
Hind part of back	7·0	0·14	6·4	1·0
Flank	8·1	0·20	13·7	1·2
Breast	9·0	0·10	21·8	1·3

Table V shows that the pH and suint content do not vary appreciably in different regions of the back, the differences between the means not being significant. However, both these values are higher on the flank than on the back, and higher still on the breast, and these differences are unquestionably significant.

Results with lambs.

In the investigation of lambs, the entire staple was used for determining grease and suint; separate samples of wool from close to the skin were taken for *p*H estimations. One batch of samples was collected from five fat lambs already bought by a local butcher for slaughter. A second series of samples was collected a week later from lambs at the College Farm, Aber. Tables VI and VII show the results obtained.

Table VI

Analyses of wool collected from fat lambs on 8 *August* 1935

Lamb no. ...	1	2	3	4	5	Mean	S.E. ±	
Shoulder	8·4	7·6	8·3	7·4	8·5	8·0	0·2	*p*H values
Mid-back	8·9	7·9	7·0	7·7	8·8	8·1	0·4	
Flank	>9·6	8·9	8·4	>9·6	9·4	(>9·2)	—	
Breast	8·9	—	9·6	—	—	(9·2)	—	
Shoulder	7·8	4·5	9·8	12·0	7·1	8·2	1·3	Suint %
Mid-back	8·7	6·4	8·0	5·7	7·9	7·3	0·6	
Flank	14·9	15·0	12·0	14·8	11·3	13·6	0·8	
Breast	6·8	13·7	15·9	14·8	11·7	12·6	1·7	
Shoulder	5·7	6·1	7·9	15·3	5·6	8·1	2·0	Grease %
Mid-back	6·2	5·2	6·7	11·0	7·7	7·4	1·0	
Flank	9·5	6·6	8·1	9·8	6·1	8·0	0·8	
Breast	3·5	4·7	7·6	7·0	4·5	5·5	0·8	

1, 2, Welsh ram lambs; 3, Southdown type ram lamb; 4, Welsh ewe lamb; 5, Welsh, wool kempy.

Table VII

Analyses of wool samples collected 15 *August* 1935

Lamb no. ...	1	2	3	4	5	Mean	S.E. ±	
Shoulder	7·8	—	7·6	7·4	—	(7·6)	—	*p*H values
Back (mid)	8·2	—	7·8	7·5	—	(7·8)	—	
Back (hind)	8·4	—	7·9	8·2	—	(8·2)	—	
Shoulder	8·8	9·9	8·1	6·8	5·5	7·8	0·8	Suint %
Back (mid)	6·9	10·2	7·0	6·1	4·4	6·9	1·0	
Back (hind)	9·8	12·0	6·7	8·1	7·9	8·4	0·6	
Flank	13·6	—	10·9	—	6·3	(10·3)	—	
Shoulder	6·3	8·7	4·3	8·6	9·1	7·4	1·0	Grease %
Back (mid)	9·0	11·6	7·1	11·4	15·2	10·9	1·4	
Back (hind)	7·9	9·4	6·6	9·7	6·0	7·9	0·8	
Flank	6·5	—	6·0	—	9·1	(7·2)	—	

All Welsh ewe lambs. No. 1, Grease uniform; No. 2, Grease uneven; No. 3, Grease uniform, wool fine; No. 4, Wool long and coarse; No. 5, Wool medium fine.

The distribution of *p*H and suint over the fleece of lambs is very similar to that found with ewes. The differences over the back are not significant and the fluctuations seem to occur in a random way; the values for the breast and flank are significantly higher than for the back. However, with lambs, the suint content on the breast was not higher than on the flank, as was the case with ewes. With regard to the grease content, it will be seen that the breast wool contains distinctly less grease than wool from the back and flank.

Comparison of struck and unstruck sheep

A further series of wool samples was collected from sheep which had been struck on the back during wet weather in the autumn, and for comparison, samples were taken from sheep of similar types which had not been infested with maggots.

Table VIII

Comparison of struck and unstruck sheep.
Samples collected on 3 October 1935

	Sheep No.	Grease %	Suint %	Remarks
Ewes:	1. Struck	12·8	2·3	
	2. ,,	14·4	2·3	
	3. ,,	9·1	0·9	
	4. Unstuck	9·5	1·1	
	5. ,,	11·5	1·4	
	6. ,,	9·6	1·3	
	Mean: struck	12·1	1·8	
	Mean: unstruck	10·2	1·3	
Lowland lambs:	7. Struck	4·4	0·5	Wool in region of strike stained yellow
	Stained wool from 7	5·9	2·0	
	8. Struck	7·9	5·5	Wool discoloured
	9. Unstruck	11·5	2·1	
	10. ,,	8·7	1·1	
	11. ,,	12·8	1·8	
	Mean: struck	6·1	3·0	
	Mean: unstruck	11·0	1·7	
Mountain lambs, wool kempy:	12. Struck	8·7	2·1	
	13. ,,	12·3	2·3	
	14. Unstruck	12·9	3·2	
	15. ,,	12·6	3·2	
	Mean: struck	10·5	2·2	
	Mean: unstruck	12·7	3·2	
Southdown cross lambs:	16. Struck	10·7	8·4	Wool discoloured and scabby
	17. Unstruck	11·2	2·1	
Mean: struck		10·0	3·0	
Mean: unstruck		11·1	1·9	

Unless stated to the contrary, the breed of sheep was Welsh and samples were taken from the middle of the back. The strikes had occurred on the back.

The results, given in Table VIII, do not show a consistent difference between struck and unstruck sheep, either in grease or suint content, though the mean suint value of all the struck sheep is considerably higher than the corresponding figure for unstruck sheep; this is mainly due to the high suint content of the wool samples from two of the struck sheep, numbers 8 and 16. The suint values are considerably lower than those previously recorded; this is doubtless due to the leaching effect of dipping. These sheep had been double dipped a few weeks before sampling; the previous analyses were made on wool from undipped sheep.

Discussion

There appears to be little known about the distribution of the yolk (suint and grease) in sheep's fleece. Bonsma & Starke(1) examined Merino sheep in South Africa and found that the suint fraction was evenly distributed over the flanks and back. Thus, the mean values obtained for five different regions lay between 19·1 and 23·0 per cent, though the wool from the belly was considerably richer in suint (mean value 35·4 per cent). The grease content was found to be more variable over the back and flanks and significantly lower in the belly wool. The results of the present investigation show that in Welsh sheep the *suint* content varies in a random way over the back, while on the flanks and breast the wool contains significantly larger amounts. This difference may be partly due to the leaching effect of rain; the back is the most exposed region and the water-soluble material probably tends to be washed from the back to the flanks. With regard to the *grease* content, the distribution appears to be fairly uniform except that the breast wool (rich in suint) was found to contain less grease than wool from the back and flanks. In addition to suint and grease, pH determinations were carried out on water extracts. It was thought that this value might prove an indication of susceptibility since blow-fly attack is usually associated with bacterial activity. The results showed that the pH values run parallel with the suint content of the wool; although the correlation is not statistically significant, nevertheless a high pH value is always associated with a high suint content and vice versa. In ewes, the reaction was found to be approximately neutral over the back and alkaline on the flanks and breast; in fat lambs the pH values tend to be more alkaline. The present investigation has been mainly concerned with the distribution of yolk over the body, but some analyses have been made of different parts of the staple. Any abnormal condition in the fleece is likely to affect the distribution of the yolk along the staple; further investigations are, therefore, being made on this subject.

The distribution of suint over the fleece requires further discussion in view of the theory of Holdaway & Mulhearn(3) that a high suint content indicates susceptibility to blow-fly strike. The present results do not support this hypothesis as the suint content of the wool proved to be relatively low on the susceptible region (back) and high on non-susceptible regions (breast and flanks). In this country (2), the back and especially the shoulder region are common sites of maggot infestation, the breast and flanks seldom being affected. In the present work, no difference in suint content was observed between the shoulder region and other parts of the back. In Australia, Holdaway & Mulhearn observed that body strike on Merinos is particularly common on the shoulder (withers). Bonsma & Starke (1) found no difference in suint content between the shoulders and the rest of the back in Merinos. There is no correlation, therefore, between the liability of certain parts of the body to blow-fly attack and the suint content of the fleece. Other factors must be responsible for the regional distribution of susceptibility. However, examination of the full analyses shows that there is considerable individual variation from sheep to sheep in the suint content of the wool. It is possible, therefore, that sheep with a large amount of suint in the fleece are more susceptible. The results obtained with wool from the same region on struck and unstruck sheep (Table VIII) are too few to admit of definite conclusions being drawn; also the samples were collected shortly after dipping. Nevertheless, they show that strike can occur on the back when the suint content is extremely low.

The conclusion of Holdaway & Mulhearn (3) that suint content is an index of susceptibility depended partly on indirect evidence and is open to serious criticism in that they carried out no suint analyses to substantiate their hypothesis. The following quotation from Holdaway & Mulhearn's paper shows how they arrived at this conclusion:

"(1) We have shown that there is a relation between yolk colour and susceptibility to weather stain and body strike: susceptibility increases with increase in intensity of yolk colour.

"(2) We have produced evidence for considering yellow yolk to be identical with golden coloration of Rimington and Stewart.

"(3) On the evidence of Rimington and Stewart, confirmed by Sutton, yolk colour is an index of sweat content.

"We must conclude, therefore, that sweat content is an index of susceptibility."

The work of Rimington & Stewart (4) mainly concerned the chemical nature of the pigment responsible for golden coloration. They gave analyses of nine wool samples, together with a brief description of their appearance, and observed "a correspondence between depth of coloration and amount of suint". This evidence led Rimington & Stewart to suspect that the pigment originates in the sweat; this is not a sufficient basis for Holdaway & Mulhearn's assumption that yolk colour can be used to measure sweat content. Sutton(5) investigated the changes in the wool yolk of forty sheep over a period of 4 months. At the time of the last sampling, "each fleece was carefully examined for various characters, including the appearance of the yolk". Sutton observed "a strong positive relationship between the intensity of the colour of the yolk and the content of water-soluble matter", and noted that this result agreed with Rimington & Stewart's conclusion, that the pigment is present in the suint. This observation was an incidental part of the work and Sutton did not apparently attempt to estimate the yolk colour on a numerical basis, as Holdaway & Mulhearn did in their investigation. Consequently, there is no evidence that yolk colour is quantitatively related to suint content. It appears, therefore, that without further work the colour of the yolk cannot be regarded as a reliable measure of the amount of suint. Since the evidence for this step in Holdaway & Mulhearn's argument is not altogether convincing, their final conclusion as to the role of sweat, although inherently probable, cannot be regarded as definitely proved.

In correlating predisposition to weather stain and strike with yolk colour, Holdaway & Mulhearn have nevertheless made a valuable advance in our knowledge of susceptibility. In this country, strike on the back seems to be often associated with abnormal colorations of the fleece and field observations are being continued on this point. It is doubtful whether the discolorations observed by us are identical with the weather stain described by Australian workers in Merinos. Much confusion still exists as to the nature and interrelation of abnormal conditions in the fleece such as weather stain, golden coloration, canary stain, dead yolk, etc. This subject merits further investigation owing to its importance in relation to wool quality and blow-fly attack. For a better understanding of these abnormalities, more information is required on the physiology of yolk secretion and the physical chemistry of the fleece under normal conditions.

Summary

1. An investigation has been made of the distribution of yolk over the fleece of Welsh sheep.

2. The suint content of the wool is relatively low on the back and high on the flanks and breast. The distribution of suint over the back appears to vary in a random way.

3. The pH of water extracts generally runs parallel with the suint content of the wool, although the correlation is not mathematically significant. The reaction is approximately neutral on the back and alkaline on the flanks and breast.

4. The grease content is fairly uniform over the back and flanks, but the wool on the breast contains relatively less grease.

5. These results are discussed in relation to Holdaway and Mulhearn's theory that sweat content is an index of susceptibility to blow-fly attack.

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A NEW APPARATUS FOR SEPARATING INSECTS AND OTHER ARTHROPODS FROM THE SOIL

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(With Plate XXXIX and 2 Text-figures)

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I. REVIEW OF METHODS PREVIOUSLY USED

In the course of some investigations now being carried on at Rothamsted on the action of soil fumigants, the ability to determine rapidly the density and nature of the soil population became essential. A scrutiny of the literature (1, 2, 3, 4, 9, 10, 12 and 13) showed that in all investigations on the insect fauna of the soil their quick separation, alive and undamaged, was a matter of great difficulty and there was no method that would quite meet our requirements.

The original primitive procedure was to crumble the soil by hand and examine it in small quantities after spreading it out on sheets of paper. With a soil containing much clay it is impossible to get it in a condition friable enough to crumble, without losing many of the smaller insects such as Collembola, and dipterous larvae, both on account of their activity in escaping and by their death on drying. As a result there is little doubt that the numbers recorded are considerably lower than the truth.

The various improvements on this primitive method may be placed in three main classes:

- (1) Voluntary movement of fauna from the soil.
  - (a) Attraction to warmth.
  - (b) Attraction to warmth aided by repulsion from light.
- (2) Separation of fauna from the soil by sieving.
  - (a) Straight sieving of soil in a semi-dry condition.
  - (b) Washing the soil through sieves with or without shaking.
- (3) Separation of fauna from the soil by flotation in water.
  - (a) Without preliminary sieving.
  - (b) On the residue after washing the soil through sieves.

(1) *Voluntary movement of fauna from the soil*

(a) *Attraction to warmth.*

Berlese (20) in 1905 devised an interesting piece of apparatus for collecting minute insects from the soil, moss and similar materials. The apparatus consists of a double-walled metal funnel with a fine-mesh sieve across the top and a tube leading to a small bottle at the bottom. The space between the two walls of the funnel is nearly filled with water which is kept warm. The material to be examined is spread out on the sieve, when the animals in it move downward through the sieve towards the warmth and away from the light into the bottle.

According to Williams (19) the greater part of the catch will come through in the first 24 hours.

(b) *Attraction to warmth aided by repulsion from light.*

Trägårdh (17, 18) made experiments with a modification of the Berlese funnel as improved by Tullgren in 1917. The soil or other material on the sieve was illuminated and heated from above by an electric lamp. The action of the lamp was twofold; one direct, resulting in the rapid movement downward of those animals that react negatively towards the light; and the more slow indirect action of the light and heat, causing a drying out of the upper layers of the material resulting in a movement of the fauna to the damper lower layers.

(2) *Separation of fauna from the soil by sieving*

(a) *Straight sieving of dry soil.*

Lane (8) in 1928, in order to facilitate the investigation of subterranean insects, particularly wireworms, used a soil sifting machine. The semi-dry soil was passed through screens with meshes of increasing fineness. He found that a screen of 12 meshes to the linear inch was sufficient to retain bigger larvae; while the smaller larvae and eggs of most of the injurious Elateridae indigenous to the North-West Pacific area of the United States could be separated from the soil by a 40-mesh screen.

(*b*) *Washing the soil through sieves with or without shaking.*

A most important step forward was made by Morris(11) when he introduced a method whereby the soil insects and other arthropods were separated by washing and stirring the soil with a strong stream of water through a series of sieves. The material left on the various sieves was then examined separately. This method gave higher numbers for the faunal population, but the quantity of soil to be examined on the various sieves was still a large fraction of the sample taken.

Morris's apparatus comprised an outer case of galvanized iron supporting three sieves of different mesh, that with the largest mesh being on the top, the intermediate size in the middle and the smallest below. A lead pipe fitted with a large spray nozzle was clamped to the upper edge of the case and was connected at the other end with a water supply; there was also an outlet at the bottom of the case. The soil to be examined was placed in the upper sieve, the water turned on and the soil thus washed out was divided into three lots, the finest particles being carried away with the waste water and the insects being retained on the various sieves according to their size.

Using Morris's method, with a 12-lb. sample of Rothamsted soil the washing occupies 50 min. to 1 hour and the subsequent examination several days.

The disadvantages of this method are:

(1) The long time and labour entailed in picking the insects off the sieves and separating them from the sandy residue.

(2) The insects are badly damaged by the agitation and friction with the stones.

(3) The constant attention required.

(4) The general uncleanliness of the process which makes it undesirable to work in an ordinary laboratory.

(5) The impossibility of using the apparatus for the large number of samples required by an experiment designed in accordance with modern statistical procedure.

Shirck(14) tried a modification of Morris's method for separating eggs and young larvae of wireworms. He designed an apparatus consisting of a wooden rack to hold a series of sieves of 10-, 30-, 40- and 50-mesh, a hose equipped with an adjustable nozzle, and a small funnel with an interchangeable screen bottom for collecting the residue from various pans after washing. The 40-mesh screen retained practically all the eggs; while the newly hatched larvae were caught by the 50-mesh screen.

Thiem (15) also constructed an interchangeable soil sieve, similar to that of Shirck, and used it successfully for separating the pupae of *Rhagoletis cerasi* L. from the soil. It consisted of a tripod supporting a funnel over which were placed four close-fitting sieves one above the other. Each sieve had an interchangeable bottom consisting of a brass screen disc soldered to the metal ring.

(3) *Separation of fauna from the soil by flotation in water*

(*a*) *Flotation without preliminary sieving.*

Daniels (5) devised a method which proved very satisfactory in North Eastern Colorado for obtaining the larvae and pupae of the potato flea beetle (*Epitrix cucumeris* Harris) in potato fields. The larvae and pupae, which were too delicate to withstand the ordinary method of sifting and washing, were floated to the surface by agitating the soil in shallow galvanized pans with water which was subsequently poured through brass screens of increasing fineness. The soil was allowed several minutes to settle so as to eliminate clogging of the screen with mud when the liquid was poured off the sample. The rubbish with the pupae and larvae were caught on a 1/20-in. screen, but the majority of the larvae were found on the 1/40-in. screen.

(*b*) *Flotation after sieving.*

Mabyn Thompson (15) at Aberystwyth after sifting the soil for a preliminary examination, took small portions and washed them through a sieve of about 0·75 mm. mesh, shaking at the same time. The residue on the sieve was then stirred with water in a dish and small organisms would float on the surface of the water. The water was poured off and the process repeated with fresh water until nothing more was found. Edwards (6) at Aberystwyth modified Thompson's procedure by using a series of three sieves 3·5 mm., 1·5 mm. and 50 meshes to the linear inch respectively.

II. DESCRIPTION OF NEW METHOD

In view of the inefficiency of the existing methods and their unsuitability for modern demands a new system of separation became necessary.

The problem was attacked in two ways:

(*a*) Direct flotation.

(*b*) Flotation by making use of the surface relationships of the insects and a liquid medium as used in the separation of minerals.

Only the first method has been developed to a state of efficiency and forms the subject of the present paper.

(1) *Preliminary trials*

The density of typical soil insects in various physiological states was determined and it was found that in no case was it higher than 1·1, even when the insect was contracted.

The next step was the selection of a liquid of the required density which would be non-toxic to the insects when the latter were immersed in it for a reasonable period, say 20–30 min. The liquid must be cheap and easily handled, and have no dispersive action on the clay in the soil.

After sundry trials a solution of magnesium sulphate in water was selected; this has the additional advantage of flocculating the clay, but some other material may be found to be equally or more suitable. Commercial calcium chloride can be used, but it precipitates the "hardness" of the tap water and the clear solution must then be decanted from the precipitate after standing. Moreover, it is more unpleasant to work with than magnesium sulphate. Water alone could be used if only Collembola, mites and other organisms with a low density were required, but in a clay soil a complete separation from the mud would not be possible.

(2) *Principle of the method*

The soil is stirred up gently with a solution of magnesium sulphate[1] (sp. gr. 1·11) in a metal cylinder and a stream of very fine air bubbles is passed up through the mixture. The air bubbles help to free the insects quickly from the soil and these rise to the top of the liquid in a froth mixed with organic debris, most of which, however, is held back by a coarse sieve placed just below the surface. The level of the liquid in the cylinder is raised by the gradual inflow of magnesium sulphate by gravity from a reservoir.

The top of the cylinder is fitted with a conical head culminating in a small rectangular opening. The froth collects in this opening and overflows down a chute into a sedimentation tank full of the magnesium sulphate solution. Any soil coming over with the froth is deposited here while the froth with its content of soil animals sweeps over the surface of the liquid on to a Buchner funnel fitted with two filter papers, No. 1 Whatman underneath, and No. 29, a black paper, on top. The liquid is filtered off and the debris given two quick washings with water. The bigger insects can be seen with the naked eye and are picked up with forceps but for smaller insects the filter paper must be cut into strips and examined under a binocular microscope. The apparatus as at present in use will take 7 lb. of soil, but works better with a smaller quantity, 5–6 lb. Once the soil is added the process of separation is almost automatic, so it would be possible to have several machines in use at the same time or one machine in use while the others were being cleaned. The limiting factor is of course the time necessary for the full examination of the debris. The quantity of residue to be examined is less than 1 per cent of the original soil, whereas with Morris's method and its modifications the quantity may be from 30 to 60 per cent or more.

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[1] Approximately 25 % solution of the commercial crystallized salt (Bath salts).

(3) *Description of the apparatus*

The apparatus (Pl. XXXIX, fig. 1 and Text-fig. 1) consists of:

(*a*) A cylinder in which the soil is mixed with the liquid.

(*b*) A conical head fitted to the top of the cylinder with a watertight connexion.

(*c*) A combined stirrer and air bubbler supporting two sieves.

(*d*) A stirring mechanism.

(*e*) An air pump and (e_1) manometer.

(*f*) A small electric motor for stirring.

(*g*) A soil sedimentation tank.

(*h*) A glass reservoir containing the solution.

(*i*) A Buchner funnel and (*j*) filter flask.

(*a*) *The mixing cylinder* (Pl. XXXIX, fig. 1, *a* and Text-fig. 1, *a*).

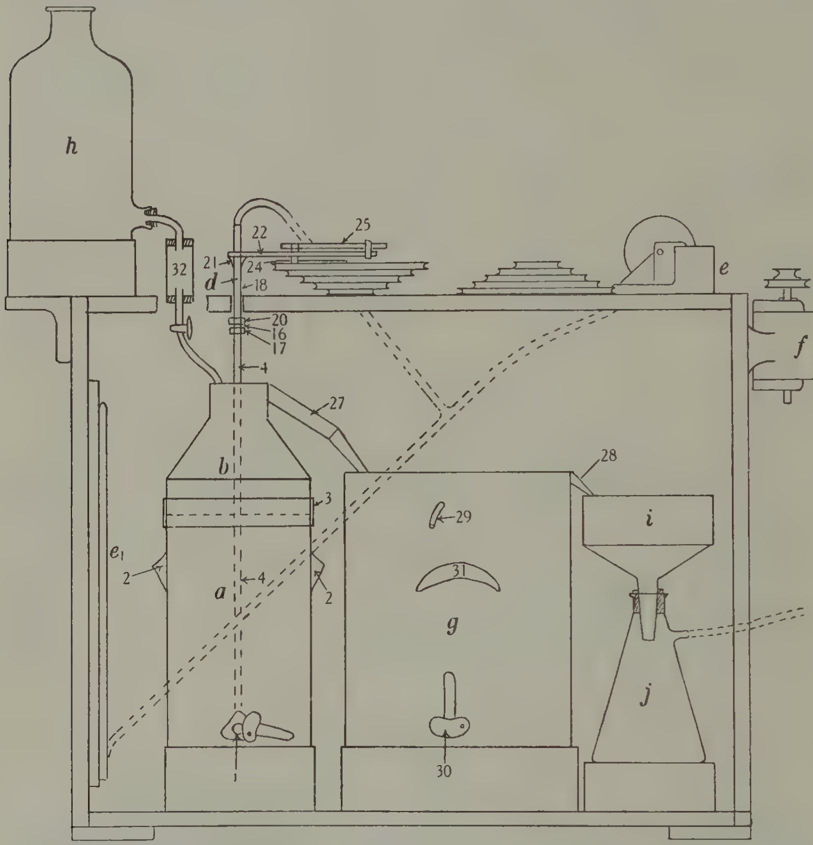
This is made of galvanized sheet iron. It is 8¼ in. in diameter and 11 in. high; it has a rim 1½ in. wide soldered on to the bottom to raise it sufficiently to allow clearance for the discharge outlet. This outlet is in the form of a "Honey-Gate" (1) and can be purchased from makers of bee-keepers' appliances. The outlet is fitted on the bottom of the cylinder and projects through the lower rim. By means of the "Honey-Gate" a quick discharge of the contents of the cylinder is obtained.

Two handles (2) are soldered opposite each other at right angles to the honey gate. In the centre of the bottom inside is soldered a small piece of brass plate with a hole to act as a bearing for the lower end of the stirrer.

(*b*) *The conical head* (Pl. XXXIX, fig. 1, *b* and Text-fig. 1, *b*).

This is made of galvanized sheet iron. A rim 1½ in. wide fits over the upper rim of the cylinder. The conical portion of the head is 4 in.

in height and culminates in a rectangle 2 in. high, 4½ in. long and 3 in. in width open at the top.



Text-fig. 1. Diagrammatic representation of the whole apparatus mounted in the wooden frame. For explanation of lettering see text.

Key to numerals used in Text-figs. 1 *and* 2

1, discharge outlet of cylinder; 2, handles; 3, rubber ring; 4, central tube of hollow stirrer; 5, hexagonal box of stirrer; 6, air tubes; 7, air outlet; 8, brass caps; 9, brass wire; 10, clip for baffles; 11, rubber stirring baffles; 12, bearing point; 16, threaded collar; 17, hexagonal nut; 18, brass tube connexion; 19, flanged tube; 20, hexagonal back nut; 21, brass boss; 22, iron strap; 23, brass pin; 24, crank; 25, connecting arm; 26, baffle plates; 27, chute; 28, lip; 29, over-flow pipe; 30, discharge outlet of tank; 31, handles; 32, tap funnel.

The watertight connexion between *a* and *b* is made thus: the upper rim of the cylinder and the rim of the conical head are each covered with a wide rubber ring and fixed in position with paint. In addition, there is another ring 3 in. wide (3) over the rubber ring on the rim of the cylinder, fixed in position by means of adhesive tape and copper wire. The upper half of this is rolled back and when the conical head is placed in position the ring is pulled over the rubber on the rim of the conical head and makes a perfectly watertight joint.

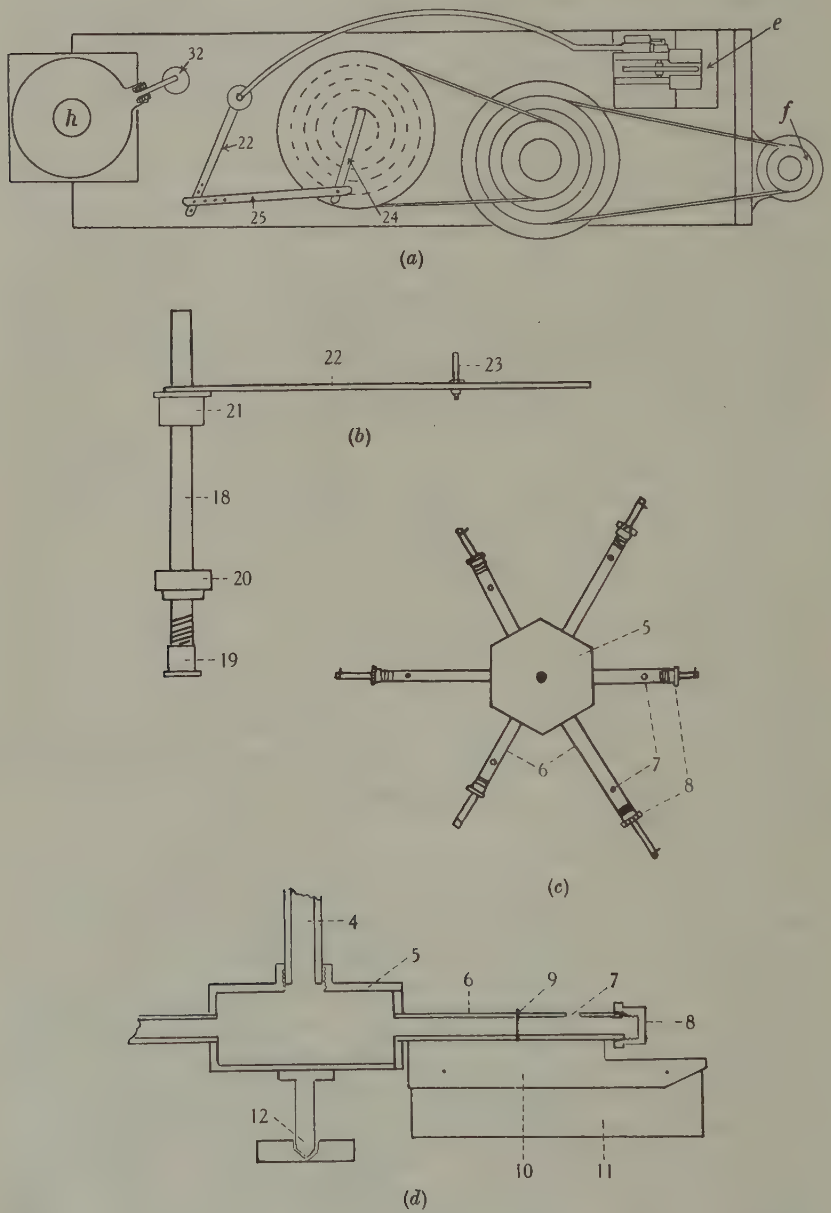
Note. An improved mixing cylinder and conical head (Pl. XXXIX, fig. 2). The cylinder is fitted with a narrow flange $1\frac{1}{2}$ in. wide. On this flange is fixed a soft rubber washer $\frac{1}{8}$ in. thick. The conical head is provided with a similar flange and rubber washer. Six bolts are fixed to the flange on the cylinder and pass through holes in the upper flange. Over each bolt is fitted a loose strip of iron 1 in. wide and 3 in. long with a hole in the middle. The flanges may then be tightened together to make a waterproof joint by means of butterfly nuts.

The connexion between the conical head and cylinder could be made with flanges and hinged bolts such as are used with autoclaves, but in that case very much heavier flanges would be required which would add considerably to the cost of the apparatus.

(*c*) *Combined stirrer and air bubbler* (Pl. XXXIX, fig. 3 and Text-fig. 2, (*c*), (*d*) and *f*).

A brass tube, $\frac{3}{8}$ in. in diameter and $23\frac{1}{2}$ in. long (4), is screwed at its lower end into a hexagonal brass box (5) $2\frac{1}{2}$ in. in diameter with faces $\frac{7}{8}$ in. deep. In the centre of each face of the hexagon a brass tube (6) $\frac{5}{16}$ in. in diameter is screwed and soldered. These tubes are alternately short and long, the short ones projecting $1\frac{3}{4}$ in. and the long ones $2\frac{1}{2}$ in. from the faces of the box.

A hole (7) $\frac{1}{8}$ in. in diameter is bored in the upper surface of each tube about $\frac{3}{4}$ in. from the outer end which is threaded and closed with a small brass cap (8). A piece of strong brass wire (9) is inserted through each tube half-way between the hole and base of the tube, and soldered in position. To the lower portion of each tube is soldered a length of channel brass (10) 3 in. long and $\frac{1}{2}$ in. wide, cut away to clear the brass caps. (If the side tubes were threaded internally, brass plugs could be used instead of the caps and this cutting away would be unnecessary.) The channel brass forms a clip holding narrow pieces of thick rubber (11) (3 in. long and $\frac{3}{4}$ in. wide) which are kept in position by two lengths of copper wire or by rivets passing through holes in the brass and rubber. The rubber strips form the stirring baffles. A piece of $\frac{5}{16}$ in. brass rod (12), $\frac{7}{8}$ in. long and pointed is screwed into a small hexagonal nut soldered on to the centre of the lower surface of the hexagonal box to act as a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(b)
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(c)
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8
12
10
11
(d)

Text-fig. 2. *a*, plan of reducing gears and stirring mechanism; *b*, elevation of stirring mechanism connexion; *c*, plan of base of stirrer and air bubbler; *d*, cross-section through base of stirrer.

bearing point. After removing the caps, the side tubes are packed tightly with cotton wool pressing against the cross-wires, and the caps replaced.

When air is pumped through the centre tube it passes through these cotton-wool pads and emerges as a stream of fine bubbles. Some little practice is needed in order to get the packing even in all the six tubes.

Experiments are being made with sintered glass and with a special type of porous stone as a substitute for the cotton wool.

The sieves (Pl. XXXIX, fig. 3). The bottom sieve is 8 in. in diameter—3 meshes to the linear inch, with a vertical rim $1\frac{1}{4}$ in. in height. The sieve is fitted with a central collar connected to the rim by means of two lengths of iron rod. The collar is bored and threaded to take a butterfly screw to fix the sieve in any desired position. The upper sieve is of the same mesh as the lower, but slightly smaller in diameter. It is dome-shaped with the convex side upwards. It is fitted with a collar and butterfly screw so that it can be fixed in a high position, out of the way, while the soil is being put into the cylinder. The lower position of the sieve is fixed by a fork made of brass with its two prongs pointing upwards. The cross bar of the sieve sits in between the prongs when the sieve is lowered. This position is such that the top of the sieve is just below the top of the cylinder.

At the top of the central tube a threaded collar (16) is fixed, $\frac{1}{2}$ in. long and $\frac{5}{8}$ in. in diameter with a hexagonal nut (17) 1 in. in diameter below it.

(*d*) *The stirring mechanism* (Pl. XXXIX, fig. 1, *d*, Text-fig. 1, *d* and Text-fig. 2, *a* and *b*).

A brass tube $7\frac{1}{2}$ in. (18) long similar to that used for the central tube of (*c*) is fitted at the lower end with a wide piece of brass tube $\frac{3}{4}$ in. long with a narrow flange (19) at the lower end screwed on and soldered in position. A hexagonal back nut (20) is a loose fit over the tube and is retained by the flange. This nut is threaded internally to engage with the threaded collar (16) of the stirrer, a lead washer being used to secure an air-tight connexion when the two hexagonal nuts are tightened with spanners.

A brass boss (21) $\frac{1}{2}$ in. wide is slipped over the tube $1\frac{3}{4}$ in. from the top and soldered in position; on this is soldered a piece of iron strip (22) $\frac{1}{8}$ in. thick, 8 in. long and $\frac{3}{4}$ in. wide rounded at each end. Holes $\frac{1}{8}$ in. in diameter are bored at $\frac{1}{2}$ in. intervals from the far end and threaded to take a brass pin (23) $1\frac{1}{8}$ in. long which extends $\frac{3}{4}$ in. above the iron strip and is held in position by means of a screw washer above and a small nut below. The best working position is when the pin is in the fifth hole from the end.

A series of reducing gears from the motor finish up with a crank (24) giving a throw of 6½ in. The connexion between the crank and the iron strip is made by an arm (25) 9 in. long with a socket at one end with four holes at ½ in. intervals at the other. One of these holes fits over the pin in the iron strip and the socket slips over the pin of the crank.

For most purposes 100–120 revolutions per minute are found to be satisfactory with the stirrer moving backwards and forwards through an angle of 50°. But these details can be altered in accordance with the motor power available and the type of soil being examined.

(*e*) *The air pump and* (e_1) *manometer* (Pl. XXXIX, fig. 1, *e* and e_1, Text-fig. 1, *e* and e_1 and Text-fig. 2 (*a*), *e*).

A Marco air pump is used. A pressure of 14–18 cm. of mercury is required to drive the air through the cotton-wool.

(*f*) *Electric motor* (Pl. XXXIX, fig. 1, *f*, Text-fig. 1, *f* and Text-fig. 2 (*a*), *f*).

A motor which develops 1/40 H.P. is used, but a slightly more powerful one would be better.

(*g*) *Soil sedimentation tank* (Pl. XXXIX, fig. 1, *g* and Text-fig. 1, *g*).

This is made of galvanized iron. It is 17 in. high, 14 in. long and 6 in. wide. Two baffle plates (26) 9 in. deep are fixed centrally across the length of the tank, 1½ in. apart. The chute (27) leading from the conical head rests on the surface of the liquid in the tank and the baffle plates serve to guide the insects and debris across the tank to the lip (28), which extends over the Buchner funnel.

On the front of the tank 1½ in. from the top a narrow pipe (29) is fixed as an overflow to allow the surplus liquid to escape. The top of the lip is fixed at a level just above that of the outlet. The discharge outlet is in the form of a honey gate (30). The tank is provided with two handles (31) one in the front and one at the back.

(*h*) *The glass reservoir* (Pl. XXXIX, fig. 1, *h*, Text-fig. 1, *h* and Text-fig. 2 (*a*), *h*).

This is an ordinary aspirator bottle with a capacity of 10 litres. The outlet is filled with a rubber stopper through the centre of which passes a glass tube bent at right angles. The lower end of the tube is inserted in a rubber stopper in the top of a small tap funnel (32) which provides a visible flow for the liquid.

(*i*) *Buchner funnel and* (*j*) *filter flask* (Pl. XXXIX, fig. 1, *i* and *j* and Text-fig. 1, *i* and *j*).

The funnel is 7½ in. in diameter and the filter flask has a capacity of 2·24 litres.

The whole apparatus is mounted in a rectangular frame 3 ft. 6 in. × 2 ft. 9 in. × 12 in. wide, with the stirring mechanism and reservoir above. The brass tube (18) of the stirring mechanism passes through a hole in the top of the frame, 1¼ in. in diameter, which is then closed by two semicircular pieces of hard wood flanged to prevent them falling through the hole. These act as the upper bearing of the stirrer.

(4) *Technique*

Mr K. D. Baweja who is using the apparatus for his investigations on soil insects finds that the following procedure gives the best results.

A soil sample 3 × 4 × 9 in. is taken with the help of iron plates designed for the purpose, and put in a bag which is closed and brought to the laboratory. It is first of all examined rapidly in an enamelled basin and all the macrofauna visible to the naked eye removed. As the soil of the Rothamsted Experimental Station is very flinty, some big stones which are likely to interfere with the subsequent treatment are separated and stirred in a vessel containing magnesium sulphate solution which is transferred to the machine during the course of the operation.

The soil after preliminary examination is poured on to the bottom sieve of the stirrer resting in the cylinder, the sieve being fixed some 4 in. above the brass box. The top sieve is then let down to its proper position and the cylinder closed with the conical head. It is now transferred to the wooden frame, connected with the air pump, and the chute of the conical head directed into the sedimentation tank so that the end of the chute is just touching the surface of the liquid. Sufficient quantity of the solution, including that from the large stones mentioned above is poured into the cylinder so as to bring its level just below the rectangular opening of the conical head. The air pump is then started and continued for 3–5 min. This helps to extricate the animals not embedded in the soil and cause them to float at once. The stirrer is now started by switching on the electric current. Uniform churning of the soil is maintained from 5 to 10 min. continuously or at repeated intervals of 3–4 min. In the process of stirring, debris along with the soil fauna starts rising up. If all the debris floats at once there is no need to work the stirrer again, but if it is found to take some time, as it frequently does, stirring is repeated once or twice as necessary. Soon after starting stirring, the solution is let into the cylinder from the reservoir and its flow regulated to about 450 c.c. per min. As the level of the solution rises all the floating material passes into the sedimentation tank. The latter is provided with a small pipe 1½ in. below the top edge connected with rubber tubing on the front side to drain off the excess of liquid. When no more debris is seen to rise, the air pump is stopped and the pipe closed. With the continuous discharge of the solution from the cylinder the level of the solution in the sedimentation tank soon begins to rise and the liquid starts floating down into the Buchner funnel. This is fitted with a white filter paper with a black one above. As the muddy solution from the cylinder passes over the sedimentation tank, the mud settles down and by the time the liquid reaches the funnel it has become nearly clear. Froth, grass blades, etc., stick to the sides of the conical head of the cylinder and the sedimentation tank and require brushing or sweeping with a rubber spatula. If the whole process is manipulated carefully very little solution is received in the funnel and this is conveniently filtered in a short time with the help of the suction pump. The residue left in the filter paper is washed rapidly with a small quantity of distilled or tap water and examined with a magnifying glass to remove the bigger organisms. The filter paper is then transferred to a glass dish and examined under a binocular microscope in strips for the more minute fauna. The whole process from taking the soil sample to the examination of residue takes from 30 to 40 min.

If churning is carried out too long, the more delicate organisms, such as aphides and thrips, are damaged; the amount of stirring is therefore according to experience.

The solution in the cylinder is drained out from the discharge outlet at the bottom and poured over a sieve (1·5 mm. mesh) into a bucket as a check to ensure the capture of larger worms that might have been missed in the preliminary examination or failed to float. But out of the observations made so far with the machine not a single specimen has thus been found.

The weight of the sample taken depends upon the moisture content of the soil. If the weather is dry it weighs from 5½ to 6½ lb., but if it is wet the weight increases to 7 and occasionally 7¾ lb. The apparatus has been dealing efficiently with the whole amount of the samples in regulated one treatment.

The total volume of liquid required for the apparatus is 47 litres:

Reservoir	10 litres
Cylinder and head	15 ,,
Tank	22 ,,

The solution may be used over and over again after standing to allow the soil to deposit. The loss is about 5 per cent per operation. Before using the solution again it must be tested with a hydrometer and if necessary brought up to strength by the addition of more magnesium sulphate.

III. RESULTS AND COMPARISON WITH OTHER METHODS

Preliminary experiments made by the writer showed that the number of insects and other animals obtained from soil with the apparatus was always much higher than that found by Morris's method.

Table I

Bubbling and mechanical stirring as compared with hand stirring—both in $MgSO_4$ *solution*

		Numbers of soil animals								
		Ladell's machine			Stirring by hand			Difference		
Series No.	Type of soil examined	In-sects (*a*)	Other inverte-brates (*b*)	Total (*c*)	In-sects (*d*)	Other inverte-brates (*e*)	Total (*f*)	In-sects (*a–d*)	Other inverte-brates (*b–e*)	Total (*c–f*)
1	Allotment fallow	104	19	123	48	7	55	56	12	68
2	Grassy plot	96	27	123	92	6	98	4	21	25
3	Grassy plot	63	17	80	52	11	63	11	6	17

Dimension of soil sample = 3 × 4 × 9 in.

Table II

Accuracy of determination on duplicate samples

		Numbers of soil animals							
		1st half soil sample (*a*)			2nd half soil sample (*b*)			Ratio of	
Series No.	Type of soil examined	Insects	Other inverte-brates	Total	Insects	Other inverte-brates	Total	(*a*)	(*b*)
1	Allotment (grass 2 years)	111	29	140	120	31	151	48·1 :	51·9
2	Allotment fallow	40	13	53	43	12	55	49·1 :	50·9

Dimension of soil sample = 3 × 4 × 9 in.

Since October 1935 the machine has been used by Mr K. D. Baweja and before adopting it for routine work he submitted it to some tests which are summarized below. Table I shows the importance of the air bubbles in separating the fauna from the soil particles. In three trials with bubbles, 123, 123 and 80 individuals were found; without bubbles, 55, 98 and 63. Table II shows the accuracy of the determinations on duplicate samples. Each soil sample was thoroughly mixed and divided

Table III. *Soil populations as determined by various methods*

Series No.	Method	Year	Locality	Type of soil examined	No. of sample examined	Average invertebrate population per acre in millions	Percentage of population: Insects	Percentage of population: Other invertebrates	Notes
1	M'Atee	1907	Washington	(*a*) Forest floor	1	1·2	24·1	75·9	—
				(*b*) Meadow land	1	13·6	80·2	19·8	About 73 % were ants
2	Cameron	1913–14	Cheshire	(*a*) Glover's meadow	11	0·8	—	—	Insects only
				(*b*) Alluvial pasture	14	1·5	—	—	—
3	Morris	1916–17	Cheshire	Lane field pasture	29	3·5	—	—	Insects only
4	Morris	1920–21	Rothamsted Exp. Sta.	(*a*) Broadbalk manured, dung	23	15·1	51·2	48·8	—
				(*b*) Broadbalk unmanured	23	5·0	49·9	50·1	—
5	Thompson	1920–21	Aberystwyth	Pasture land	20	19·5	43·8	56·2	—
6	Edwards	1925–6	Aberystwyth	Pasture	20	27·2–44·6	48·3–69·2	51·7–32·8	—
7	Ladell	1935–6	Rothamsted Exp. Sta.*	Allotments:					
				(*a*) Fallow	10	60·6	79·2	20·8	—
				(*b*) Grassy, 2 years	3	121·4	79·1	20·9	—
				(*c*) Broadbalk:					
				(1) Manured, dung (Feb. 1936, warm and moist)	1	106·1	80·8	19·2	—
				(2) Manured, dung (Feb. 1936, ground frozen)	1	40·8	80·8	19·2	—
				(*d*) Broadbalk unmanured (Feb. 1936, ground frozen)	1	25·6	85·7	14·3	

* These figures were obtained by Mr K. D. Baweja working at Rothamsted in 1935–6.

Table IV. *Insect populations as found by the present method in comparison with that of Morris*

Series No.	Method	Year	Locality	Type of soil examined	No. of individuals per acre in millions: Collembola	Thysanura	Orthoptera	Thysanoptera	Hemiptera	Lepidoptera	Coleoptera	Diptera	Hymenoptera	Psocoptera	Unidentified eggs	Total	Notes
1	Morris	1920–1	Rothamsted Exp. Sta.	Broadbalk manured, dung	2·39	0·11	0·01	0·02	0·01	0·03	0·79	1·41	2·96	—	—	7·73	Average of 23 observations
2	,,	,,	,,	Broadbalk unmanured	0·69	0·04	0·01	0·02	0·00	0·04	0·37	0·54	0·71	—	—	2·42	Average of 23 observations
3	Ladell	1935–6	,,	Allotments (*a*) Fallow	21·11	0·1	0·2	—	13·71	—	1·78	5·60	0·16	0·01	5·18	47·85	Average of 10 observations
4	,,	,,	,,	(*b*) Grass 2 years	91·79	—	—	0·89	0·52	—	1·41	0·5	0·16	—	0·35	95·62	Average of 3 observations
5	,,	1936	,,	Broadbalk manured	58·55	18·29	—	—	—	—	3·11	3·66	—	—	2·09	85·70	One observation only Weather warm and moist. February
6	,,	,,	,,	Broadbalk manured	27·18	0·52	—	—	—	—	1·04	3·14	—	—	1·04	32·92	Soil frozen. February
7	,,	,,	,,	Broadbalk unmanured	19·86	—	—	—	—	—	1·57	0·52	—	—	—	21·95	Soil frozen. February

into two portions which were treated separately in the machine. The agreement between the two halves is remarkable. In one, 48·1 per cent of the fauna was found in one half and 51·9 per cent in the other; in the second sample, 49·1 per cent was found in one half and 50·9 per cent in the other. Table III shows the total animal population as determined by various methods on different types of soil. The figures are not fully comparable but they do show that by the use of the new apparatus very high figures are obtained. Table IV details the insect populations found by the present method, in comparison with Morris's figures obtained with Rothamsted soils. Morris found 7·73 million insects per acre on the dunged plots on Broadbalk. Mr Baweja's two observations are 85·7 million on a warm moist day in February and 32·9 million on a day in the same month when the soil was frozen hard. These observations on Broadbalk will be continued throughout the year in order to obtain a fuller and more accurate comparison. The average of ten observations (from October to February) on the fallow allotments gives a figure of 47·9 million insects and on the plots that had been grass covered for 2 years 95·6 million insects per acre.

These figures are sufficient to indicate the high efficiency of the apparatus described in this paper.

IV. SUMMARY

1. The published methods for the separation of insects and other animals from the soil are briefly described.

2. None of these methods being sufficiently rapid or efficient for soil fumigation investigations, a new method has been devised.

3. The principle of the method is flotation by a dense liquid (a solution of magnesium sulphate sp. gr. 1·11) aided by stirring of the soil and a stream of fine air bubbles passing from the bottom upwards through the mixture of soil and solution. This produces a froth which contains all the animals, and by raising the level of the liquid in the cylinder the froth is swept over into a tank filled with magnesium sulphate solution; here is deposited any soil that has been carried over. The clear solution is then passed on to a Buchner funnel where the insects and other animals are retained. A black filter paper is used in order to show up Collembola and other colourless organisms.

4. The apparatus has many advantages:

(*a*) *Rapidity*. The separation taking 30–40 min. from taking the soil sample of 6–7 lb. weight.

(*b*) *Cleanliness.* The operation can be carried out in an ordinary laboratory.

(*c*) *Efficiency.* All the organisms are concentrated in a small volume of residue, less than 1 per cent of the original soil, and are undamaged.

(*d*) *Non-toxicity.* The great majority of the insects come through alive, so that eggs and larvae may be bred out for purposes of identification.

5. A full account is given of the technique employed. The solution may be used again and again as the clay settles very quickly and the clear liquid may be syphoned or poured off.

6. Very high figures have been obtained for the soil population. These are much in excess of those recorded by most other workers with agricultural soils; for example, from fallow land 60·6 million animals per acre were recorded and from new grass land, 121·4 million per acre.[1]

V. ACKNOWLEDGEMENTS

The writer is much indebted to Dr C. B. Williams for his advice and many helpful suggestions and to Mr P. S. Milne and Mr D. M. T. Morland for their practical assistance in constructing the apparatus. The overflow pipe was an improvement proposed by Mr J. Glasgow. Thanks are also due to Mr K. D. Baweja for his thorough testing of the apparatus and for permission to include some of his results.

The whole of this work has been made possible by a grant from Messrs Imperial Chemical Industries, Ltd.

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[1] Ford (7) found a population of 276·6 million per acre (both surface and soil animals) in a meadow near Oxford "covered with a thick mat of vegetation and rotting grass".

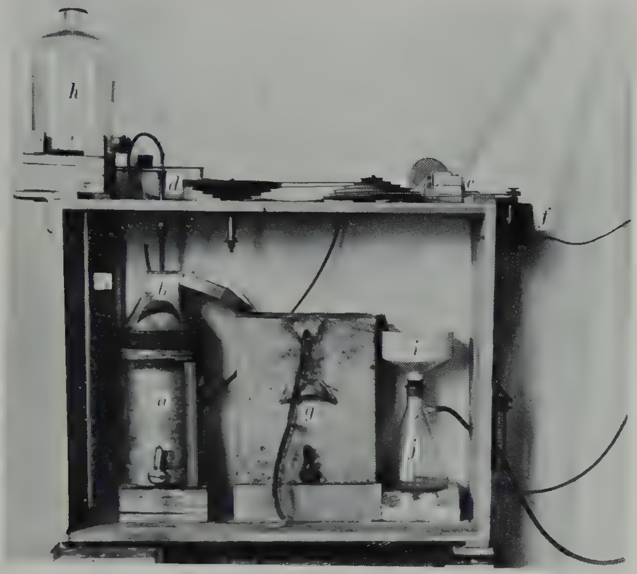

Fig. 1

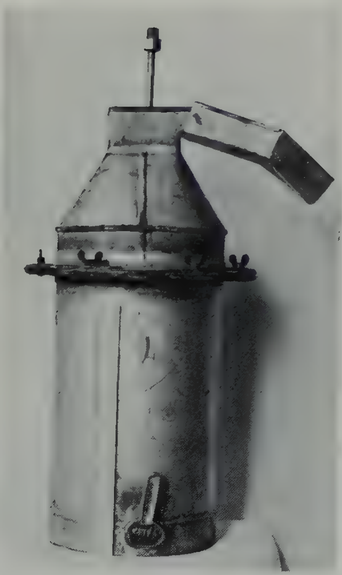

Fig. 2

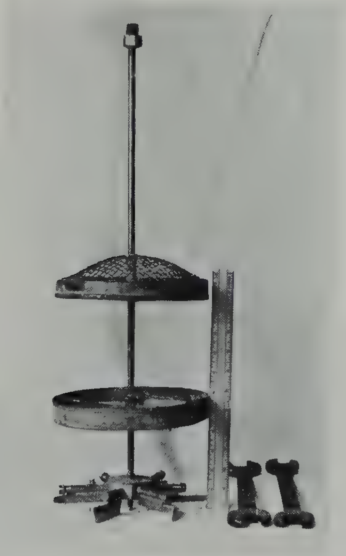

Fig. 3

LADELL—A New Apparatus for Separating Insects and other Arthropods from the Soil
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EXPLANATION OF PLATE XXXIX

Fig. 1. General view of the apparatus. *a*, mixing cylinder; *b*, conical head; *d*, stirring mechanism; *e*, air pump; e_1, manometer; *f*, electric motor; *g*, soil sedimentation tank; *h*, glass reservoir for solution; *i*, Buchner funnel; *j*, filter flask.

Fig. 2. Improved cylinder with conical head. The watertight joint being made by two flanges with rubber washers in between. The central tube of the stirrer and air bubbler is seen above the rectangular opening of the head of the cylinder.

Fig. 3. The stirrer and air bubbler. Showing the sieves and brass box with air tubes and stirring baffles. The two spanners are for use on the hexagonal nuts, one on the central tube and the other on the tube of the stirring mechanism.

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THE PROBLEM OF THE EVALUATION OF ROTENONE-CONTAINING PLANTS

II. *DERRIS ELLIPTICA*, *DERRIS MALACCENSIS* AND THE "SUMATRA-TYPE" ROOTS

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(With 2 Text-figures)

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Introduction

In previous work reported in the first paper of this series (10) an examination was made of the chemical and insecticidal properties of two species of derris root at present in extensive use as insecticides, namely *Derris elliptica* and *D. malaccensis*. In this investigation, certain chemical factors were determined, and these in turn were then correlated with the insecticidal potencies of the roots to *Aphis rumicis*.

Of the limited number of samples examined, those of *Derris elliptica* were found to have a higher proportion of rotenone to total ether extract than was the case with the specimens of *D. malaccensis*, and Georgi & Teik in Malaya, working with roots of similar origin and kind, have also referred to this fact (3). From the analysis of twelve samples of derris from the Dutch East Indies, Spoon (7) found that those of *D. malaccensis* gave high ether extract but low rotenone, while the *D. elliptica* roots, showing less ether extract, were relatively richer in rotenone. It does not, however, seem possible to distinguish with certainty between roots of *D. malaccensis* and *D. elliptica*, by a consideration only of the relative proportions of rotenone to ether extract, since exceptional roots have been recorded. Thus Koolhaas (6) reports that a sample of *D. malaccensis* was found to contain 4·3–4·6 per cent of rotenone, amounting to 31–35 per cent of the total ether extract of the root, figures that may be thought to be more indicative of *D. elliptica*. More work is needed upon the relative proportions of rotenone to ether extract in authentic specimens of the two species before a general rule can be established.

A variety of derris is now in use, which although toxic, yields no rotenone by the normal method of separation of the rotenone-carbon tetrachloride complex. To such a root the name "Sumatra-type" has been given by Cahn & Boam (1) who show that rotenone is present in a "hidden" form, and may be induced to separate by the addition of an excess of the pure compound. It is a matter of some importance to characterize this type of root with greater precision in order to determine whether it approximates in chemical and physical properties to the more commonly known varieties of *D. elliptica* or *D. malaccensis*, or whether it is likely to prove to be a new species. In addition it is desirable that a suitable method of chemical evaluation, applicable to all types of derris, should be available in order to ascertain the degree of variation in the insecticidal efficiency of these roots due to cultivation or to varying climatic conditions.

We have shown (*loc. cit.*), in comparisons of *D. elliptica* with *D. malaccensis*, that neither the rotenone nor the ether extract could be relied upon to give a correct measure of the relative insecticidal activities of the roots, but that the determination of the mixed dehydro compounds, or of rotenone plus the dehydro compounds determined on the residual rotenone-free resins, gave a better assessment. In the present work we have compared, both chemically and biologically, a "Sumatra-type" root with samples of *D. elliptica* and *D. malaccensis* in order to ascertain to what extent the determination of the dehydro compounds, or of other chemical factors, will correctly evaluate the toxicity of the "Sumatra-type" root relative to the other two species examined.

EXPERIMENTAL

The "Sumatra-type" root (our No. W 170) was one kindly sent to us by the Cooper Technical Bureau, while the samples of *D. elliptica* and *D. malaccensis* used were two samples remaining from the tests carried out during the previous year, and reported upon in the first part of the present series. They were *D. elliptica* var. Sarawak creeping (our No. W 149) and *D. malaccensis* var. Sarawak erect (our No. W 151). The samples were all in the finely ground condition. The "Sumatra-type" root showed a crude rotenone content, determined by the "hidden" rotenone technique of Cahn & Boam (*loc. cit.*) of 1·95 per cent, this figure being reduced to 0·54 per cent of purified rotenone on trituration of the complex with alcohol saturated with rotenone. The *D. elliptica*

Table I

The rotenone contents of "Sumatra-type" root (W 170), D. malaccensis (W 151) *and* D. elliptica (W 149)

			% of fresh root	
Sample	Date of analysis		Rotenone (crude)	Rotenone (purified)
"Sumatra-type" W 170	Oct. 1935	Ether, CCl_4, Cahn & Boam's "hidden" rotenone	2·01	—
	Feb. 1936	Ether, CCl_4, Cahn & Boam's "hidden" rotenone, recrystallization from CCl_4	2·07	0·47
	Mar. 1936	Ether, CCl_4, Cahn & Boam's "hidden" rotenone, Cahn & Boam's trituration	1·95	0·54
D. malaccensis Sarawak erect	Apr. 1933	Ether, CCl_4, recrystallization from alcohol	2·54	1·83
W 151	Oct. 1935	Ether, CCl_4, Cahn & Boam's "hidden" rotenone, recrystallization from alcohol	2·70	1·81
	Feb. 1936	Ether, CCl_4, recrystallization from CCl_4	3·13	1·87
	Mar. 1936	Ether, CCl_4, Cahn & Boam's trituration	2·84	1·93
D. elliptica Sarawak creeping	Apr. 1933	Ether, CCl_4, recrystallization from alcohol	5·08	3·83
(W 149)	Oct. 1935	Ether, CCl_4, Cahn & Boam's "hidden" rotenone, recrystallization from alcohol	5·07	4·05
	Feb. 1936	Ether, CCl_4, recrystallization from CCl_4	5·26	4·07

In the recrystallization of the complex, allowance was made in each case for the solubility of rotenone in the solvent used.

gave figures of 5·08 per cent of crude, and 3·83 per cent of recrystallized rotenone, while the *D. malaccensis* figures were 2·54 and 1·83 per cent (Table I). It is of interest to note that there was no significant falling off in the rotenone contents of samples W 149 and W 151 on keeping the finely ground powders from April 1933 until March 1936 in tins at room temperatures in the region of 20° C. (see Table I).

INSECTICIDE TESTS

Biological trials using the three samples were carried out during the summer of 1935. Trouble was experienced in the early part of the summer by the incidence of disease in the stock of insects. Later, however, this was satisfactorily eliminated, but the number of biological trials it was possible to carry out was considerably reduced. The "Sumatra-type" root was compared alternately with the *D. elliptica* root W 149 and with the *D. malaccensis* root W 151. A weighed portion of each root was extracted with ether, the dried resin dissolved in alcohol, and a series of dilutions with 0·5 per cent saponin solution prepared. The alcohol content of each solution tested was adjusted to 5 per cent by volume, and the apparatus used was that previously described (9). Adult apterous females of *Aphis rumicis* were used as test subjects, and comparisons only made between tests carried out on the same day. As in the previous work (*loc. cit.*), we have taken the percentages of badly affected, moribund and dead insects (B, M, and D per cent) observed on the second day's examination as indicating the toxic effects. The concentrations employed, expressed as mg. of root per litre, with the observed mortalities, are given in Table II and are plotted against the probit values in Figs. 1 and 2. The comparison of the "Sumatra-type" root with *D. elliptica* W 149 was made on 31 July, and the comparison with the *D. malaccensis* W 151 root on 12 August 1935. In the comparison of the "Sumatra-type" root with W 149, the former gave 50 per cent of badly paralysed and dead insects at a concentration equivalent to 685 mg., and the *D. elliptica* W 149 at a concentration equivalent to 238 mg. root per litre of spray fluid. In the later comparison of the "Sumatra-type" root with *D. malaccensis*, the concentrations giving 50 per cent "mortality" were equivalent to 763 and 338 mg. of root per litre respectively. The test insects used thus show a greater resistance in the later than in the earlier trials. In Table II, columns 2–8, are given the concentrations tested expressed in terms of various chemical determinations, to which detailed reference will be made later (pp. 886–893).

Table II

Comparison of the toxicities of the "Sumatra-type" root W 170, *with* D. elliptica W 149 *and* D. malaccensis W 151. *Insect used* Aphis rumicis. *Fivefold replication, ten insects at a time. Results* 2 *days after spraying*

	Concentrations (mg./1000 c.c.) tested, in terms of							Toxicity results			
Sample	Root	Purified rotenone	Total dehydro compounds	Rotenone + dehydro compounds on resin soluble in ether after potash extraction	Resin soluble in ether after potash extraction	"Toxic" resin ("rotenone equiv.") soluble in ether after potash extraction	Rotenone + "deguelin concentrate"	No. of insects	Insects paralysed B + M + D %	S.E. % ±	Probits of B + M + D
"Sumatra-type" root W 170	1250	6·8	102	20	49	39	39	50	96	4·0	6·751
	1000	5·4	82	16	39	31	31	50	86	6·0	6·080
	750	4·1	61	12	30	23	23	50	66	8·1	5·413
	625	3·4	51	10	25	19	19	49	36·7	6·6	4·660
	500	2·7	41	8	20	16	16	49	16·3	5·2	4·018
D. elliptica W 149	353	13·5	38	37	53	53	38	50	92	5·8	6·405
	282	10·8	30	30	42	42	31	40	55	6·6	5·126
	212	8·1	23	22	32	32	23	50	42	5·8	4·798
	176	6·8	19	19	26	26	19	50	18	2·0	4·085
Control (saponin and alcohol solution)	—	—	—	—	—	—	—	50	0	—	—
D. malaccensis W 151	547	10·0	43	36	49	45	43	50	91·3	3·7	6·360
	437	8·0	35	29	39	36	34	50	78·3	6·3	5·782
	328	6·0	26	22	29	27	26	49	33·5	6·5	4·574
	273	5·0	22	18	25	23	21	50	30·4	4·0	4·487
	219	4·0	17	15	20	18	17	49	17·9	6·6	4·081
"Sumatra-type" root W 170	1250	6·8	102	20	49	39	39	50	95·7	2·4	6·717
	1000	5·4	82	16	39	31	31	50	69·6	3·7	5·513
	750	4·1	61	12	30	23	23	49	40·1	8·4	4·749
	625	3·4	51	10	25	19	19	50	34·8	10·9	4·609
	500	2·7	41	8	20	16	16	50	15·2	3·7	3·972
Control (saponin and alcohol solution)	—	—	—	—	—	—	—	50	8	3·7	—

The B + M + D percentages are given allowing for control figure of 8 %. Standard errors calculated on percentages before allowing for control.

Comparison of toxicities of "Sumatra-type" root W 170 and *D. elliptica* W 149

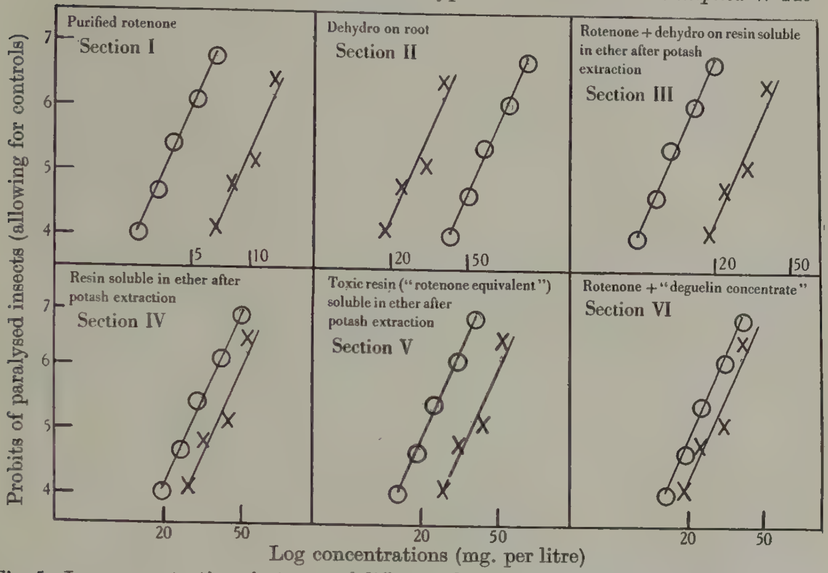

Fig. 1. Log concentrations in terms of different chemical values plotted against probits of badly paralysed insects (B, M and D per cent). ○ "Sumatra-type" root W 170 × *D. elliptica* W 149

Comparison of toxicities of "Sumatra-type" root W 170 and *D. malaccensis* W 151

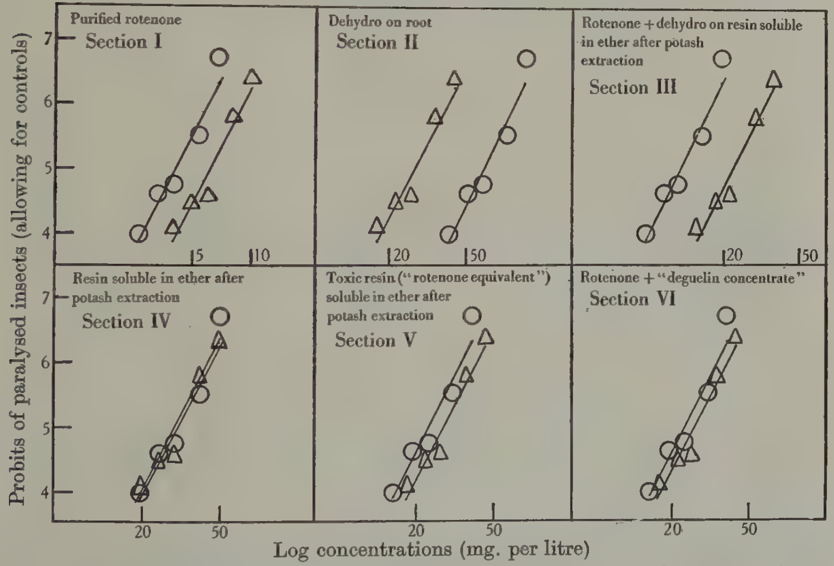

Fig. 2. Log concentrations in terms of different chemical values plotted against probits of badly paralysed insects (B, M and D per cent). ○ "Sumatra-type" root W 170, Δ *D. malaccensis* W 151.

CHEMICAL EXAMINATION OF THE ROOTS

Rotenone. In the determinations of the rotenone content of the "Sumatra-type" root the "hidden" rotenone method outlined by Cahn & Boam (*loc. cit.*) has been used, following failure to induce crystallization on dissolving in carbon tetrachloride the resin extracted from the root by ether. In the trituration of the product it was found preferable to filter at 0°C., using alcohol saturated with rotenone at this temperature. The purification of the complex resulted in a much reduced figure, the crude product appearing to be contaminated by resin. Reference to Table II and Figs. 1 and 2 shows that the estimation of purified rotenone does not give a correct assessment of the relative insecticidal efficiencies.

Ether extract. The determination of the ether extract was made by extracting 5 g. of root with anhydrous ether, the resin being dried to constant weight in an electric oven kept at 100° C. On the resin so obtained, the percentage of methoxyl content was determined by the method of Clark (2), and from it that of the root calculated. The ether extract or methoxyl figures (Table III) clearly do not assess the activities of the roots.

Table III

Analytical data for "Sumatra-type" root W 170,
D. malaccensis W 151 *and* D. elliptica W 149

	% of fresh root		
	"Sumatra-type" W 170	*D. malaccensis* W 151	*D. elliptica* W 149
Moisture	6·70	6·48	6·42
Rotenone (crude)	1·95	2·54	5·08
Rotenone (purified)	0·54	1·83	3·83
Ether extract	18·33	18·56	17·50
Methoxyl (on ether extract)	2·48	2·64	2·60
Benzene extract	19·02	19·42	17·94
Methoxyl (on benzene extract)	2·51	2·70	2·61
Total dehydro compounds	8·16	7·90	10·75

Dehydro compounds. The dehydro compounds were determined on the resin extracted by ether from 25 g. of the "Sumatra-type" root by the modification of the method of Takei *et al.* (8) given in paragraph 2 on p. 587 of our previous communication (*loc. cit.*). The mixed dehydro product was equivalent to 8·16 per cent of the root, and melted at 201° C. This is of the same order as the yield of the mixed dehydro compounds obtained by the same method from the resin of *D. malaccensis* W 151 (7·90 per cent). From biological trials, however, the *D. malaccensis* was seen to be approximately twice as toxic as the "Sumatra-type" root. Thus it is evident that in the comparison of these two roots the determination of the dehydro compounds is inadequate as a means of obtaining the relative activities, the "Sumatra-type" being overvalued by this means with respect to the *D. malaccensis*. The results of the analyses are given in Table III.

Treatment of the resins with dilute alkali, and the determination of dehydro compounds on the resulting fractions

It was decided at this stage to carry out a fractionation of the resins by means of dilute alkali, in order to determine to what extent the presence of toxicarol in the resin influenced the figure for the mixed dehydro compounds obtained. The use of dilute aqueous alkali in removing the toxicarol fraction of the resin has been suggested by Haller & La Forge (4) and utilized by Jones *et al.* (5) in their work on the chemical evaluation of derris. In the first fractionations carried out, portions of 50 g. each of the "Sumatra-type", *D. malaccensis* and *D. elliptica* roots were extracted with ether, and with the latter two the ether was removed and the residue taken up with carbon tetrachloride as in the normal rotenone determination. The rotenone separating was filtered, and recrystallized from alcohol, the alcoholic mother liquors being retained. From the carbon tetrachloride solutions the solvent was removed as completely as possible *in vacuo*, and the residues dissolved in ether. The ether solutions of the resins from each root were extracted three times in a separating funnel with 50 c.c. of 5 per cent aqueous potash solution. The alkaline extracts were combined, washed with ether, the ether layers separated and added to the main ether solution. After washing with water, the ether was distilled and the resins dissolved in absolute alcohol, the alcoholic filtrates from the earlier rotenone recrystallizations being incorporated at this point. The yields of dehydro compounds from the ether-soluble resins were then determined.

In the case of the "Sumatra-type" root, the alkali treatment of the ether solution of the resin resulted in the production of a copious yellow precipitate, and this also appeared in the alkaline extract of the *D. malaccensis* resin. The extract of the *D. elliptica* resin, however, showed no precipitate, the alkaline layer appearing brown-red in colour. The precipitates were filtered, washed, and taken up in ether after treatment with dilute hydrochloric acid. The clear brown solutions after removal of the yellow precipitates and the alkali extract of the *D. elliptica* resin were also acidified, and the freed resins taken up in ether. The solvent was removed in each case and the residue, after solution in alcohol, subjected to the dehydro process as outlined. The determinations on the potash-soluble fractions were unsatisfactory, mixed resinous products resulting. The yields of the dehydro compounds from the ether-soluble resins and from the resins recovered from the yellow precipitates, expressed as percentages of the fresh root, are given in Table IV. In addition, the percentage recoveries of dehydro compounds from the weights of resins actually submitted to the dehydro process are given in brackets.

Table IV

Dehydro compounds determined on fractions of resins following alkali treatment in ether solution

	"Sumatra-type" W 170		*D. malaccensis* W 151		*D. elliptica* W 149	
	% of root	% of resin	% of root	% of resin	% of root	% of resin
Dehydro compounds on total ether extract	8·16	(44·5)	7·90	(42·6)	10·75	(61·4)
Recrystallized rotenone removed	—	—	1·64	—	4·36	—
Dehydro compounds on resin soluble in ether after potash extraction	1·56	(43·3)	5·01	(54·6)	6·12	(58·6)
Dehydro compounds on resin from yellow precipitate extracted by potash	3·58	(33·2)	1·73	(48·0)	—	—

In the case of the "Sumatra-type" root, the yield of the dehydro compounds determined on the resin soluble in ether after the potash treatment, amounted to 1·56 per cent of the root, and presumably resulted from the rotenone and deguelin present. By reference to the methoxyl figures given in Table V, the resin soluble in ether after potash treatment in the case of the "Sumatra-type" root was less rich in "toxic" material than were the corresponding resins from the other two roots, but even so the recovery of dehydro compounds from it (43 per cent) was unduly low.

With the *D. malaccensis* and the *D. elliptica*, the combined figures for the separated rotenone and the dehydro compounds determined on the ether-soluble resins after the removal of toxicarol, amounted to 6·65 and 10·48 per cent of the roots respectively. The method of determination of the dehydro derivatives of the active principles is not a quantitative one, particularly so in the case of the "toxicarol" resin, where a yield of somewhat less than 50 per cent is obtained. In addition, the unsuitability of the method as a means of assessing the activity of the "Sumatra-type" root *vis-à-vis D. malaccensis* and *D. elliptica* is clearly demonstrated by

Figs. 1 and 2 (sections 2 and 3). In section 2 of each figure, the equivalent log concentrations of the dehydro compounds determined on the total ether extracts are plotted against the probits of the badly paralysed insects. In each case, the lines for the *D. malaccensis* and *D. elliptica* roots lie to the left of that for the "Sumatra-type" root, indicating that the latter, relative to the other two, is less toxic than its dehydro figure of 8·16 per cent would lead us to expect, if we assume the dehydro figures provide a correct measure of activity. In this case, the dehydro figure clearly overestimates the activity of the "Sumatra-type" root. When, however, we take the values obtained for the separated rotenone plus the dehydro compounds determined on the residual toxicarol-free resins, there is a reversal in the positions of the lines, and now the activity of the "Sumatra-type" root is undervalued relative to the other two roots.

Fractionation of the resins in ether solution

This was carried out using as before 5 per cent aqueous potash solution, the amounts of "toxic" constituents in each fraction being assessed by means of methoxyl determinations carried out upon the products isolated. With each of the three types of root under consideration, two portions of 5 g. were extracted with anhydrous ether, the solvent removed from one of the duplicate tests, the resin dried to constant weight at 100° C., and the methoxyl content determined. The second ether solution (50 c.c.) in each case was extracted successively with 10, 5 and 5 c.c. of 5 per cent aqueous potash, the alkaline extract being washed as before with ether. The resins from the precipitates and alkali-soluble fractions were recovered by acidification with dilute hydrochloric acid and solution in ether. The ether solutions were washed, dried with anhydrous sodium sulphate, the solvent removed, and the resins dried at 100° C. until of constant weight (Table V). Methoxyl determinations were then made upon the fractions obtained, special care being taken to ensure that all traces of ether had been removed; small amounts of ether were found to be tenaciously retained, it being necessary to heat the resins *in vacuo* in order to remove final traces of solvent. From the methoxyl values of the fractions, the contents of active principles of each, based in the case of the ether-soluble resins upon the methoxyl content of rotenone and deguelin of 15·74 per cent, and in the case of the alkali-extracted resins upon the methoxyl content of toxicarol of 15·12 per cent, were determined. The figures for the "toxic" constituents of each fraction, expressed as percentages of the fresh root, are given in Table VI.

Table V

Fractionation of the resins, in ether solution, by means of potash solution

	"Sumatra-type" W 170	*D. malaccensis* W 151	*D. elliptica* W 149
Total ether extract	18·33 (13·53)	18·56 (14·13)	17·50 (14·87)
Resin soluble in ether after potash extraction	3·93 (12·41)	8·95 (14·48)	14·94 (15·70)
Resin extracted and precipitated by potash	6·27 (15·40)	4·16 (14·91)	—
Resin extracted and soluble in potash	7·19 (11·83)	4·50 (10·92)	2·30 (6·94)

Figures expressed as percentages of fresh root. Percentage of methoxyl in each fraction given in brackets.

Table VI

"Toxic" constituents in each fraction based upon the methoxyl contents

	"Sumatra-type" W 170	*D. malaccensis* W 151	*D. elliptica* W 149
"Rotenone plus deguelin" (soluble in ether after potash extraction*)	3·10	8·23	14·90
"Toxicarol" equivalent (extracted and precipitated by potash†)	6·27	4·10	—
"Toxicarol" equivalent (extracted and soluble in potash†)	5·63	3·25	1·06
Total "toxic" constituents	15·00	15·58	15·96

Figures expressed as percentages of fresh root.

* Based on methoxyl content of rotenone and deguelin of 15·74 per cent.

† Based on methoxyl content of toxicarol of 15·12 per cent.

With the "Sumatra-type" and *D. malaccensis* roots 95 per cent, and with the *D. elliptica* 98·5 per cent of the total ether extract were recovered in the fractions separated. The combined "toxic" constituents for each root based upon the methoxyl contents of the resins separated were of the order of 82–90 per cent of the total ether extract figures.

We again see from the probit-concentration curves (Figs. 1 and 2) both for the ether-soluble resins after potash treatment (section 4), and also for the calculated "rotenone plus deguelin" contents of these fractions (section 5), that the "Sumatra-type" root is undervalued with respect to the other types. It would appear that the material extracted by alkali, or a related compound, is playing some part in the insecticidal action of the root.

The effect of the substitution of saturated baryta solution for the potash upon the amounts of resin remaining in solution in the ether was determined. The ether extract from 5 g. of each root was extracted three times successively with 10 c.c. of baryta, the ether layers dried as before, and the amounts of resin in solution determined. The figures for the ether-soluble resin for the "Sumatra-type" root, *D. malaccensis* and *D. elliptica* (3·76, 9·12 and 14·80 per cent of the fresh roots respectively) were in close concordance with the amounts of resin remaining in the ether after the use of potash.

The fractionation of the resins with 5 per cent aqueous potash was repeated, using in this case benzene as solvent. Duplicate portions of 5 g. of each root were extracted with benzene, one test being used in each case for the determination of the percentage of extract. Fractionation of the second solution was carried out, using successively 10, 5 and 5 c.c. of aqueous potash solution. In no case was a precipitate formed, the alkali extracts appearing as brown solutions. The total alkaline extracts of each root were washed with benzene, which was added to the main benzene solution. The benzene extracts were dried, and the amounts of resin in solution determined. It was found that, after acidification and solution in ether, only a relatively small percentage of the resin was extracted by the potash from the benzene extracts of the three types of root treated. The results are given in Table VII.

Table VII

Fractionation of the resins, in benzene solution, by means of potash solution

	"Sumatra-type" W 170	*D. malaccensis* W 151	*D. elliptica* W 149
Total benzene extract	19·02 (13·22)	19·42 (13·92)	17·94 (14·57)
Resin soluble in benzene after potash extraction	14·54 (14·23)	15·34 (14·58)	15·42 (15·61)
Resin extracted and soluble in potash	3·02	2·87	1·84

Figures expressed as percentages of fresh root.
Methoxyl content as percentage of each fraction given in brackets.

Further fractionation of the resins that had remained soluble in the benzene on extraction with potash was then carried out by dissolving them in 50 c.c. of ether and extracting successively with 10, 5 and 5 c.c. of saturated baryta solution. By this procedure the yellow precipitates, separating before in the case of the "Sumatra" and *D. malaccensis* roots on extracting with potash or baryta from ether solutions, were again obtained, with a corresponding reduction in the amounts of resin remaining in solution in the ether layers. The resins extracted by the alkali were, as before, recovered by solution in ether after acidification with dilute acid. The results are given in Table VIII. Methoxyl determinations on the fractions separated, where carried out, are given in brackets.

Table VIII

Fractionation of the resins soluble in benzene after potash extraction by means of baryta, using ether as solvent

	"Sumatra-type" W 170	*D. malaccensis* W 151	*D. elliptica* W 149
Resin soluble in benzene after potash extraction	14·54 (14·23)	15·34 (14·58)	15·42 (15·61)
Resin soluble in ether after baryta extraction	3·55 (12·48)	7·32 (14·84)	13·34 (15·50)
Resin extracted and precipitated by baryta	9·51 (14·83)	6·27 (14·55)	0·76
Resin extracted and soluble in baryta	0·57	0·38	0·41

Figures expressed as percentages of fresh root.
Methoxyl content as percentage of each fraction given in brackets.

The potash treatment of the benzene solutions of the resins has effected a partial purification, as judged by the methoxyl values, although some loss of methoxyl has occurred as well. The amounts of "toxic" resin, calculated from a methoxyl content of rotenone and deguelin of 15·74 per cent, remaining soluble in the ether after the two alkaline treatments, although slightly lower, are of the same order as the amounts given in Table VI. When the results given in Table VIII are compared with those in Table V it is seen that the baryta extraction has precipitated a larger proportion of the material extracted by alkali than has potash. In no case did the baryta-soluble material exceed 1 per cent of the root. The outstanding effect obtained in these tests was the inability of the potash to effect the separation of the potassium salt of toxicarol or of its precursor, from a benzene solution of the "Sumatra" or *D. malaccensis* resins.

Fractionation of the resins using petroleum ether as precipitant

It is known that rotenone, deguelin and toxicarol are almost insoluble in petroleum ether. This fact was utilized in an attempt to purify the resins by precipitation of the toxic constituents from concentrated ether solution by means of petroleum ether. Our experience, based upon a number of trials, was that this procedure did not effect a sufficiently quantitative separation of the active principles to justify its use for purposes of evaluation.

Preparation of "deguelin concentrates" after petroleum ether extraction of the roots

Haller & La Forge (*loc. cit.*) have shown that although the solubility of the toxic constituents of derris in petroleum ether is low, prolonged extraction will separate a resin containing a high proportion of the active principles, contaminated with extraneous material relatively small in amount by comparison with extracts using other solvents. They state that such an extract is particularly suitable for the examination of the non-crystallizable constituents. We have used prolonged extraction with petroleum ether in a Soxhlet apparatus in a further endeavour to prepare, as quantitatively as possible, "deguelin concentrates" from the three types of root under investigation.

Portions of 20 g. of the "Sumatra", *D. malaccensis* and *D. elliptica* roots were extracted with petroleum ether, with rapid refluxing, for 55 hours, the root material being taken out and mixed at frequent intervals to ensure penetration of the solvent. The petroleum ether was removed, the extracts dissolved in a little ether, the solutions seeded, and placed in an ice-chest for 2 days. Some crystals separated, particularly in the *D. elliptica* extract and these were removed. The ether solutions were then extracted with dilute potash, washed with water, dried over sodium sulphate and after concentration to a small bulk, again placed in the ice-chest for 5 days. A further amount of rotenone was deposited from the *D. malaccensis* extract. The weights of the resins remaining in solution in the ether were then determined (Table IX).

Table IX

Preparation of "deguelin concentrates" following petroleum ether extraction of the roots

	% of fresh root		
	"Sumatra-type" W 170	*D. malaccensis* W 151	*D. elliptica* W 149
Rotenone separating from ether solution	0·45	1·62	1·88
Resin ("deguelin concentrate") remaining in ether solution	2·65	6·19	8·97

The combined figures for the separated rotenone and resin remaining in solution in the ether were 3·10, 7·81 and 10·85 per cent of the roots examined. The activity of the "Sumatra-type" root is again somewhat undervalued by the figure of 3·10 per cent, both with respect to the

D. malaccensis (7·81 per cent), and to the *D. elliptica* (10·85 per cent) (see Table II and Figs. 1 and 2, section 6).

NATURE OF THE RESIN RECOVERED FROM THE MATERIAL PRECIPITATED BY POTASH FROM AN ETHER EXTRACT OF THE "SUMATRA-TYPE" ROOT

On acidification of the yellow precipitate and extraction with ether a yellow resin resulted, which has not so far been induced to crystallize[1]. The resin had a methoxyl content in close agreement with the theoretical value for inactive toxicarol (15·12 per cent), and was optically active, a solution in benzene showing a laevo-rotation. On addition of potash in methyl alcohol to such a solution, an immediate change to a dextro-rotation, followed by a decline in rotation, took place, and this reaction has been found to be characteristic of the "Sumatra-type" and *D. malaccensis* resins. It will be discussed in more detail in Part III of the present series (11). When the resin was dissolved in alcohol and refluxed with addition of potash, yellow crystals soon separated, and these, on recrystallization from acetic acid, melted at 224° C. Inactive toxicarol, obtained from "Sumatra-type" resin by the method of Cahn & Boam (*loc. cit.*) melted at 221° C. The insecticidal activity of the resin has not yet been determined, but further investigation of this interesting derivative is called for, as it appears to be rich in a precursor of the inactive toxicarol.

THE EFFECT OF THE REMOVAL OF TOXICAROL UPON THE SEPARATION OF ROTENONE FROM CARBON TETRACHLORIDE SOLUTIONS OF "SUMATRA-TYPE" AND *D. MALACCENSIS* RESINS

We have found, with a "Sumatra-type" root from which no rotenone separates by the normal procedure, that after the removal of the preponderating toxicarol by alkali treatment of the ether extract, the naturally occurring rotenone separates readily on taking up the residual resin in carbon tetrachloride. The product furthermore appears to be but little contaminated by resin. As opposed to this, quite considerable loss in apparent rotenone takes place on purification of the complex obtained by the Cahn & Boam "hidden" rotenone technique.

Duplicate portions of 4 g. each of the resin extracted by ether from the "Sumatra-type" root W 170, were taken. To one was added 10 c.c. of carbon tetrachloride, the resin dissolved, the solution cooled, seeded and kept in the ice-chest for 2 days. No crystals separated. One gram of pure rotenone was added and the determination of the "hidden" rotenone carried out by the method of Cahn & Boam (*loc. cit.*). The carbon tetrachloride mother liquor, on cooling and standing, deposited a further small amount of complex, and this was added to the main bulk of crystals. The crude complex, giving a figure of 1·95 per cent of "hidden" rotenone in the root, was purified by trituration, the purified rotenone being 0·54 per cent of the root. The second portion of resin, in 50 c.c. of ether solution, was extracted three times with 20 c.c. of 5 per cent potash, the alkaline extracts washed with ether, the combined ether solutions washed with water, dried over anhydrous sodium sulphate, the ether removed, and the residual resin taken up in 10 c.c. of warm carbon tetrachloride. After standing overnight, the first crystals were filtered, the mother liquor concentrated, and the final yield of complex obtained. The rotenone content calculated from the complex so obtained, was 0·47 per cent of the root. On purification by trituration this figure fell to 0·40 per cent. In the case of the complex separated by the "hidden" rotenone method, the product was dirty yellow in appearance, while the crystals separating after alkaline treatment were almost colourless.

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[1] An optically active crystalline compound possessing insecticidal properties was isolated from the resin shortly after this paper was sent to press.

The tests were repeated using a second "Sumatra-type" root (our No. W 180). The Cahn & Boam method was carried out as before on 4 g. of resin, while for the alkali treatment, followed by separation of the complex, the resin extracted by ether from 50 g. of root was used. In this instance, the complex was purified by recrystallization from alcohol, a correction being made for the rotenone retained by the solvent.

In previous rotenone determinations on the *D. malaccensis* W 151 root the crude complex had been highly coloured, and had shown a considerable loss in apparent rotenone on purification. The effect of alkali pretreatment of the resin of this root was therefore determined. In this case, the use of the "hidden" rotenone technique was not necessary, the complex, equivalent to 2·84 per cent of "crude" rotenone, separating overnight. On purification by alcoholic trituration the product lost much of its yellow colour, and a purified rotenone figure of 1·93 per cent of the root resulted. The "crude" rotenone determined on the duplicate portion of resin from which the toxicarol had been removed by extraction with alkali, was 1·96 per cent, the product being colourless. On purification by trituration, this figure was reduced to 1·70 per cent of rotenone in the root. The results are tabulated in Table X.

In the case of the "Sumatra-type" roots either the presence of large amounts of resinous material rich in toxicarol prevents the separation from carbon tetrachloride solution of the rotenone present, or potash treatment removes some other inhibitor of crystallization. The complex, which subsequently separates readily, is then obtained in an amount agreeing relatively closely with the figure obtained for the purified product by the normal method.

Table X

Separation of rotenone from carbon tetrachloride solutions of the resins with and without removal of toxicarol

	% of fresh root		
		Purified rotenone	
	Crude rotenone %	%	M.P. (°C.)
"Sumatra-type" W 170:			
Ether, carbon tetrachloride, Cahn & Boam's "hidden" rotenone method	1·95	0·54	(161)
Ether solution extracted with potash, carbon tetrachloride	0·47	0·40	(158)
"Sumatra-type" W 180:			
Ether, carbon tetrachloride, Cahn & Boam's "hidden" rotenone method	2·15	0·67	(161·5)
Ether solution extracted with potash, carbon tetrachloride	0·89	0·66	(159)
D. malaccensis W 151:			
Ether, carbon tetrachloride	2·84	1·93	(158·5)
Ether solution extracted with potash, carbon tetrachloride	1·96	1·70	(159)

More work is needed upon the suitability of alkali as a means of reducing the concentration of the inhibitor of rotenone crystallization. There is the possibility of the alkali causing a loss, by degradation, of the rotenone present, while oxidation products may be formed during the process of extraction. In this connexion, we have observed small, though varying amounts of a pale yellow material, melting in the region of 208° C., separating from the ether layer. It may well be, however, that such a pretreatment, suitably controlled, could form the basis of a standard method of rotenone determination applicable to the main types of derris root.

Discussion

The work described was carried out in an endeavour to characterize more definitely on a chemical basis, the "Sumatra-type", *D. malaccensis* and *D. elliptica* roots, with a view to their evaluation by chemical means. The "Sumatra-type" root examined contained small amounts of rotenone and deguelin, while some 70 per cent of its resin was removed from an ether solution by treatment with potash. From the fraction precipitated by the alkali an optically active resin, appearing rich in toxicarol, was obtained. The root was characterized by the inability of the rotenone to separate directly from a carbon tetrachloride solution of its resin, and this inhibition appeared to be due to the preponderance of the material extractable by potash.

The *D. malaccensis*, from its chemical properties, occupied a position intermediate between the "Sumatra-type" root and the *D. elliptica*, in that it contained greater amounts of both rotenone and deguelin, with a corresponding decrease in the content of alkali-extractable resin. With the *D. malaccensis*, this appeared to be insufficient in amount to inhibit the crystallization of the rotenone. The separation of rotenone is apparently dependent upon the relative proportion of rotenone to other resins in the carbon tetrachloride solution, and takes place when the proportion of rotenone to the inhibiting material is sufficiently high, a condition achieved either by the addition of the pure compound, as in Cahn's method, or by the removal of the non-crystallizable resin by treatment with alkali. Further work is needed, however, on this question before the factors influencing the separation of the complex are fully understood.

The *D. elliptica* differed markedly from both the "Sumatra-type" and *D. malaccensis* roots, in that very little material was extracted by potash from an ether solution of its resin, the extract showing no formation of precipitate. The rotenone present separated readily, and showed little contamination by resin. The "Sumatra-type" root is thus much more closely akin to the *D. malaccensis* than it is to the *D. elliptica* root.

Throughout the work, we have evidence that, although the insecticidal activity of the toxicarol as it occurs in the root is probably not comparable with that of rotenone, the preponderating amount present plays a definite part in the final toxic value of the "Sumatra-type" root. In all methods of attempted evaluation after the removal of the toxicarol the activity of the "Sumatra-type" root was undervalued, relative to the other roots, by the estimates obtained of the residual combined rotenone and deguelin. This undervaluation of the "Sumatra-type" root was greater in the comparison with the *D. elliptica* than it was in the tests with the *D. malaccensis*, a root of more similar type.

The dehydro method as a means of assessing the toxicity of the "Sumatra-type" root is inadequate and it would appear that the evaluation of derris types will only be possible when more is known of the nature and activities of the precursors in the root of the various crystalline and relatively inactive derivatives that have been isolated.

SUMMARY

1. The determinations of purified rotenone, ether extract, dehydro compounds, ether-soluble resin after potash treatment, and of the rotenone plus "deguelin concentrates" are each shown to be inadequate as a means of assessing the relative insecticidal activities of the "Sumatra-type", *D. malaccensis*, and *D. elliptica* roots.

2. The toxicarol present in the "Sumatra-type" derris appears to play a small but definite part in the insecticidal activity of the root.

3. The resin recovered from the material precipitated by potash from an ether extract of the "Sumatra-type" root is optically active, and appears to be rich in the precursor of inactive toxicarol.

4. Rotenone, if present, will separate readily from a carbon tetrachloride solution of "Sumatra-type" resin from which the toxicarol has been removed. The possibility of a standard method of rotenone determination, dependent upon suitable pretreatment of the resins, is suggested.

We wish to express our indebtedness to the Copper Technical Bureau for their kindness in supplying us with samples of the "Sumatra-type" root. We also wish to thank Dr S. Krishna of the Forestal Department of Northern India for valuable assistance.

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THE PROBLEM OF THE EVALUATION OF ROTENONE-CONTAINING PLANTS

III. A STUDY OF THE OPTICAL ACTIVITIES OF THE RESINS OF *D. ELLIPTICA*, *D. MALACCENSIS* AND THE "SUMATRA-TYPE" ROOTS

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(With 4 Text-figures)

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Introduction

One of the chief difficulties met with in attempting the assessment of the insecticidal value of the rotenone-containing plants, by either chemical or physical methods, lies in the great variety and complexity of types or strains. Not only is this true when different genera or species are considered, but also, within the same species marked contrasts have been noted. Henderson's (3) investigations bear witness to the wide botanical variation in habit of growth and leaf form of the *Derris* species. The chemical complexity of the extracts derived from these plants is no less confusing, and specimens of any one of the *Derris* species are known to vary widely in their chemical characteristics. Parts I (6) and II (5) of this series of papers deal with the more chemical aspects of the problem. We propose in this section to present a study of the optical activities of the extracts of three types of derris with the object of demonstrating as far as possible the extent and nature of this variation. Although only three samples are dealt with, it is hoped that the investigations described may prove a contribution to the differentiation of types or strains in this genus.

The importance of the study of optical rotations for this purpose is emphasized by the fact that the precursors of some of the isolated crystalline derivatives, e.g. deguelin and toxicarol, probably exist in the resins in an optically active state although the isolated crystalline products are devoid of this property. The optical rotation of rotenone in solution in various solvents has been investigated by Jones & Smith (4). It is the only constituent of a toxic nature the rotation of which is accurately known, and unquestionably it plays an important rôle in determining the optical activity of resins in which it occurs. The use of a rotation method for purposes of discrimination, evaluation and standardization is hardly warranted, without further information as to the parts played by other constituents of the derris resins. The "Sumatra-type" root presents certain possibilities in an investigation of this type, as it has been shown by Cahn & Boam (1) to contain large amounts of toxicarol. On the other hand, it is indicated in Part II of this series of papers that *D. elliptica* may be practically free from this constituent. *D. malaccensis* would seem to rank between these two in the chemical make-up of its resins. Inactive toxicarol is almost devoid of toxicity to insects and requires for its separation a treatment of the resin with alkali. A comparative study of the rotation of extracts of these three types of root and the changes induced by the addition of alkali, might make it possible to ascertain more exactly the relationship between them.

EXPERIMENTAL

The optical rotations of benzene solutions of rotenone and three different types of resin

Experiments were carried out to select solvents for extracting the resins and determining the rotations. Ether has the advantage of low-temperature extraction, but is difficult to use in the polarimeter. Thus it becomes necessary to take off the ether which these resins retain with some tenacity, and it was found that heating rotenone or derris resins at 100° C. slightly lowered their specific rotations. The rotations were therefore determined directly on the benzene extracts. This solvent has, moreover, the advantage of giving higher rotations for rotenone than many others, and with the resins, solutions of which are often so dark coloured as to transmit very little light, makes possible the use of low concentrations. The benzene extract of the finely powdered root was made up to a known volume at 20° C. and the solid residue determined in an aliquot part. Dilutions of known concentrations at 20° C. were then prepared. The rotations were determined at 20° C. in a polarimeter, reading in circular degrees, using a 10 cm. tube fitted with a water jacket for controlling the temperature. An electrically heated sodium lamp provided the illumination. In Table I are given the data obtained for the three types of derris root examined and for rotenone; they are plotted in Fig. 1. Results obtained by Jones & Smith (4) for rotenone in benzene solution are also plotted for comparison. The two sets of data are concordant, indicating that the rotenone used was of corresponding purity. Our sample was recrystallized several times from absolute alcohol and had a melting-point of 163–164° C. All the resins are laevo-rotatory, and the rotations when plotted against the concentrations are not strictly linear. The specific rotations similarly plotted fall approximately on parallel straight lines inclined to the axis of concentration (Fig. 1).

Table I

Rotations of benzene solutions of extracts of derris root of different types

Name	Conc. of resin in g./100 c.c.	Conc. as g. of root/100 c.c.	Rotation α_D^{20}	Specific rotation $[\alpha]_D^{20}$
Sumatra type	17·802	100	−9·82	−55
Benzene extract	14·244	80	−8·35	−59
Extract = 17·802 %	8·545	48	−5·45	−64
	4·272	24	−2·90	−68
	2·136	12	−1·50	−70
D. malaccensis	19·058	100	−14·17	−74
Benzene extract	15·246	80	−11·88	−78
Extract = 19·058 %	9·148	48	−7·65	−84
	4·574	24	−4·04	−88
	2·287	12	−2·08	−91
D. elliptica	17·406	100	−14·61	−84
Benzene extract	13·925	80	−12·14	−87
Extract = 17·406 %	8·355	48	−7·71	−92
	4·177	24	−4·01	−96
	2·089	12	−2·05	−98
Rotenone	(1) 5·0	—	−11·21	−224
Benzene solution	1·0	—	−2·32	−231·5
	(2) 4·6730	—	−10·49	−224·5
	2·2655	—	−5·20	−229·5
	1·0635	—	−2·46	−231·0

The question arises whether the rotatory power can be correlated with the insecticidal activity. The order of toxicity of these three samples of root judged at the median lethal dose for *Aphis rumicis* is "Sumatra-type" 1 < *D. malaccensis* 2·2 < *D. elliptica* 2·9. When the rotations of the resins for the three samples are placed in order of magnitude at some equivalent concentration of the roots, e.g. 100 per cent, the samples are placed in the same order as that given by our toxicity data (Part II, p. 884), assuming that the rotations are a measure of toxicity the order is 1 $S<1{\cdot}4\ M<1{\cdot}5\ E$ (where S = Sumatra-type, M = *D. malaccensis* and E = *D. elliptica*). This quantitatively overvalues the "Sumatra-type" relatively to the other two. It should be noted, however, that the proportionality of the rotations changes with concentration.

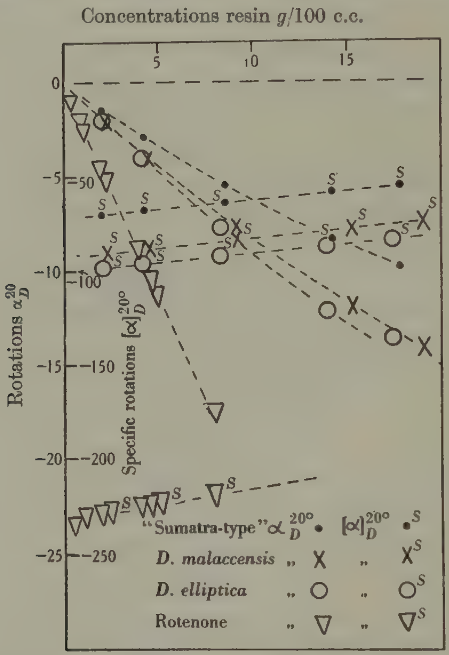

Fig. 1. Rotations and specific rotations of the benzene solutions of rotenone, and the resins of three types of derris root.

Specific rotations in benzene of the ether extracts of the roots

Portions of 10 g. each of the "Sumatra-type", *D. malaccensis* and *D. elliptica* roots were extracted with ether, the solvent removed and the resins dried as completely as possible in an oven at 60° C. The resins, the weights of which varied between 1·74 and 1·91 g. were dissolved in benzene, and the volumes adjusted to 25 c.c. at 20° C. The specific rotations of the "Sumatra-type", *D. malaccensis* and *D. elliptica* resins were −57·8°, −85·6° and −95·3° respectively. These figures increased to −65·5°, −90·8° and −100·7° on dilution of 10 c.c. of each solution to 25 c.c. with benzene, and redetermination of the rotations.

Further portions of each root were extracted with ether, and the solutions (about 50 c.c. in bulk) extracted successively with 20, 20, 10 and 10 c.c. of 5 per cent aqueous potash. The alkaline extracts were washed with ether, the ether solutions for each root combined, washed with water, dried over sodium sulphate, the solvent removed and the specific rotations of the ether-soluble resins determined in benzene solution. The weights of resin for the "Sumatra-type", *D. malaccensis* and *D. elliptica* roots were 0·410, 0·959 and 1·545 g., and the specific rotations determined at 20° C., were −72·6°, −112·6° and −94·5° respectively. The specific rotations, on dilution as before, were increased to −76·2°, −115·3° and 99·1°.

In the extraction of the "Sumatra-type" and *D. malaccensis* roots, the yellow precipitates formed in the alkaline layers were filtered and washed with water, and the resins, freed on acidification with dilute hydrochloric acid, taken up in ether. The ether solutions were washed free from acid, dried over sodium sulphate, and the solvent removed. The specific rotations of the resins were then determined in benzene solution. They were as follows: resin from the yellow precipitate from the "Sumatra-type", root −62·7° and for the resin from the *D. malaccensis* precipitate −67·5°. Haller & La Forge (2) state that the precipitate formed on potash treatment of an ether solution of derris resin consists of the difficultly soluble potassium salt of toxicarol. On recovery of the potassium-free material from this by acidification and extraction with ether, the product, which so far we have not succeeded in obtaining in the crystalline condition[1], is shown to be optically active and laevorotatory in benzene.

We have, in addition, separated toxicarol from a "Sumatra-type" root by the method of Cahn & Boam (*loc. cit.*). The crystalline product melted at 221° and had a low solubility in ether and benzene. A 2 per cent solution in chloroform showed no optical rotation.

We have not been able to trace any simple correlation of the insecticidal powers of these roots and the rotations of either the crude resins or their constituent fractions. Much more information is needed on the rotations of the fractions of the derris resins and their relative toxicities.

[1] See, however, footnotes, pp. 894 and 915.

The change of rotation on adding caustic potash in methyl alcohol to benzene extracts of derris root

Apart from rotenone, the several crystalline derivatives, e.g. deguelin and toxicarol isolated from derris root have been found optically inactive. There is some evidence to show that in the process of extraction these compounds have either been derived from some precursor or have undergone a process of racemization during extraction. As alkali was employed during the process it seemed advisable to ascertain what effect it had upon the rotation of the resins derived from the samples of derris root of the three types under examination. The benzene extracts used for the determination of the rotations at different concentrations (Table I) were employed. Aliquot parts (5 and 10 c.c.) were taken at 20° C., and known amounts of normal caustic potash solution in methyl alcohol were added; in addition, an equivalent volume of methyl alcohol was added to a further set in order to ascertain its effect, if any, upon the rotation. The higher concentrations of the resins became too dark under alkali treatment, and the investigations were therefore limited to the two lowest concentrations of the benzene extracts. The following distinguishing letters have been employed:

	Dilution	Resin g./100 c.c.
"Sumatra-type"	S.C.	4·272
	S.D.	2·136
D. malaccensis	M.C.	4·574
	M.D.	2·287
D. elliptica	E.C.	4·177
	E.D.	2·089

In addition, the effect was determined of the addition of methyl alcohol and the standard solution of caustic potash in methyl alcohol upon the rotations of different concentrations of rotenone and of the various fractions, isolated by caustic potash extraction of ether solutions of the resins, all in solution in benzene.

In Table II we have given all the salient data obtained for the "Sumatra-type". The equivalent of caustic potash to be added was calculated, assuming the resin to have the same molecular weight as rotenone. This assumption is not strictly valid, but it gave a rough approximation of the amount of caustic potash to be added and experience showed that within certain limits the amount of alkali added, although having a distinct bearing on the initial change of rotation, had only a slight one upon its subsequent rate of change.

In the table also the specific rotation of the mixture of methyl alcohol with the benzene solution of the resin is given, the resulting concentration of resin being calculated on the assumption that there was no change in volume; the corresponding specific rotation of this concentration in benzene was determined from the graph in Fig. 1. It will be noted that the addition of methyl alcohol to the benzene solution of the "Sumatra-type" resin results in a reduction of the laevo-rotation. In a preliminary experiment on this resin, in which attempts were made to dissolve it in methyl alcohol, a portion of the resin was only slightly soluble in this

Table II

Effect of addition of caustic potash in methyl alcohol upon benzene extracts of "Sumatra-type" root

Dilution S.D. 2·136 g. resin/100 c.c. =12 g. root/100 c.c.
$\alpha_D^{20} = -1{\cdot}50°$. $[\alpha]_D^{20} = -70{\cdot}2°$.
2 c.c. methyl alcohol added to 5 c.c.
Conc. after addition of methyl alcohol =1·526 g. resin/100 c.c.
α_D^{20} after addition of methyl alcohol after
5 min. $= -0{\cdot}69°$ $[\alpha]_D^{20} = -45{\cdot}2°$
3 hours $= -0{\cdot}73°$ $[\alpha]_D^{20} = -47{\cdot}8°$
22 hours $= -0{\cdot}75°$ $[\alpha]_D^{20} = -49{\cdot}2°$
α_D^{20} for equivalent conc. in benzene $= -1{\cdot}07°$ $[\alpha]_D^{20} = -70°$.

Dilution
S.C. 4·273 g. resin/100 c.c.
$\alpha_D^{20} = -2{\cdot}9°$. $[\alpha]_D^{20} = -67{\cdot}9°$.
4 c.c. methyl alcohol added to 5 c.c.
Conc. after addition of methyl alcohol = 2·3736 g./100 c.c.
α_D^{20} after 4 hours $= -0{\cdot}81°$.
$[\alpha]_D^{20} = -34°$.
α_D^{20} equiv. conc. in benzene $-1{\cdot}65°$. $[\alpha]_D^{20}$ $-6{\cdot}95°$.

Equivalents of potash. Resin calculated as having mol. wt. 394.

	1			2			3			1		
Time min.	$[\alpha]_D^{20}$ °	$[\alpha]_{t_1}-[\alpha]_{t_2=330}$ °	k*	$[\alpha]_D^{20}$ °	$[\alpha]_{t_1}-[\alpha]_{t_2=205}$ °	k*	$[\alpha]_D^{20}$ °	$[\alpha]_{t_1}-[\alpha]_{t_2=115}$ °	k*	$[\alpha]_D^{20}$ °	$[\alpha]_{t_1}-[\alpha]_{t_2=195}$ °	k*
1	+185	+116	—	—	—	—	+208	+131	—	—	—	—
2	—	—	—	+200·5	+116	—	—	—	—	+177	132	—
3	175	106	0·045	—	—	—	203	126	0·022	170	125	0·055
5	172	103	0·030	190	105·5	0·032	196	119	0·025	158	113	0·052
10	160	91	0·027	175	90·5	0·031	180	103	0·028	130	85	0·055
15	147	78	0·028	161	76·5	0·032	166	89	0·028	110	65	0·054
20	138	69	0·027	151	66·5	0·031	154	77	0·028	96	51	0·053
25	132	63	0·025	142	57·5	0·031	146	69	0·027	84	39	0·053
30	125	56	0·025	137	52·5	0·028	135	58	0·028	75	30	0·053
35	118·5	49·5	0·025	130	45·5	0·028	128	51	0·028	68	23	0·053
40	113	44	0·025	126	41·5	0·027	122	45	0·028	64	19	0·051
45	109	40	0·024	—	—	—	—	—	—	—	—	—
55	—	—	—	113	28·5	0·026	109	32	0·026	54	9	0·051
60	100·5	31·5	0·022	—	—	—	—	—	—	—	—	—
70	—	—	—	106	21·5	0·025	96	19	0·028	49	4	0·051
75	93	24	0·023	—	—	—	—	—	—	—	—	—
85	—	—	—	98	13·5	0·026	88	11	0·030	46	1	0·059
105	85	16	0·019	—	—	—	—	—	—	—	—	—
115	—	—	—	93	8·5	0·023	77	0	—	—	—	—
145	—	—	—	89	4·5	0·023	—	—	—	—	—	—
195	75	6	0·016	—	—	—	—	—	—	45	0	—
205	—	—	—	84	0	—	—	—	—	—	—	—
330	69	0	—	—	—	—	—	—	—	—	—	—
	Mean k =0·026 Mean k/c.c. methyl alcohol =0·013			Mean k =0·0285 Mean k/c.c. methyl alcohol =0·014			Mean k =0·0275 Mean k/c.c. methyl alcohol =0·0135			Mean k =0·053 Mean k/c.c. methyl alcohol =0·0133		

* Natural logs were used in calculating k.

solvent and some of the fractions obtained when dissolved in methyl alcohol were dextro-rotatory in contrast with their solutions in benzene which were laevo-rotatory.

The addition of caustic potash in methyl alcohol causes a definite colour darkening of the solution and an instantaneous change from a laevo- to a dextro-rotation. The rotations then decline at first rapidly and afterwards more slowly. The readings were taken for the first and second minutes after mixing and afterwards every 5 min. until the change became so slow as to require a longer period for accuracy. Only one reading could be taken at these 5 min. intervals, so rapid was the early rate of change, but for the 15 min. intervals five readings, and for 30 min. and longer intervals ten readings were taken, the mean of each set being given in Table II. After a period the change became very slow and the solution darkened to such an extent as to render further readings impossible. In no instance did the dextro-rotation fall to zero with time, but approached a value which could be regarded as the end-point of the reaction. We are unable to state the nature of the product giving rise to this residual dextro-rotation or whether it was present in constant amount during the course of the decline in the rotatory power. It was considered preferable to take the final reading as the equilibrium point indicating a cessation of the main reaction. This value was therefore deducted from the other readings for purposes of calculating the velocity coefficient assuming the reaction to be unimolecular. The curves obtained by plotting the specific rotations against the time interval only approximate to the semi-logarithmic type, the value for the velocity coefficient $\frac{1}{t_2-t_1} \log \frac{a-x_1}{a-x_2}$, in which, after deducting the final reading, a is the initial rotation and x_1 and x_2 the changes of rotation after time t_1 and t_2, tends to decline with time, but, with many of the curves is approximately constant at their middle portion. For the lower concentrations of resin the mean velocity coefficient is practically independent of the amount of caustic potash added. The velocity coefficient for dilution S.C. is approximately double that found in the tests given by dilution S.D. Later work, the results of which are given below, demonstrated that the increase in velocity is not due to the increase in concentration of the resin, but to the amount of methyl alcohol added with the caustic potash. At the foot of Table II we have given the values for the ratio Mean k/MeOH c.c.[1]. It is approximately the same in every case, namely 0·013–0·014.

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[1] I.e. the number of c.c. of methyl alcohol added to 5 c.c. of the benzene solution of the resin.

The data for the similar treatment of *D. malaccensis* are given in Table III.

Table III

The effect of addition of caustic potash in methyl alcohol upon benzene extracts of D. malaccensis *root*

Dilution M.D. (2·287 g. resin/100 c.c. = 12 g. root/100 c.c.). $\alpha_D^{20} = -2{\cdot}08°$. $[\alpha]_D^{20} = -90°$.		Dilution M.C. (4·574 g. resin/100 c.c. = 24 g. root/100 c.c.). $\alpha_D^{20} = -4{\cdot}04°$. $[\alpha]_D^{20} = -88{\cdot}3°$.
2 c.c. methyl alcohol added to 5 c.c.	4 c.c. methyl alcohol added to 5 c.c.	4 c.c. methyl alcohol added to 5 c.c.
Conc. after adding methyl alcohol = 1·634 g./100 c.c.	Conc. after adding methyl alcohol = 1·27 g./100 c.c.	Conc. after adding methyl alcohol = 2·541 g./100 c.c.
$[\alpha]_D^{20}$ after adding methyl alcohol = −69°.	$[\alpha]_D^{20}$ after adding methyl alcohol = −61°.	$[\alpha]_D^{20}$ after adding methyl alcohol = −59·0°.
$[\alpha]_D^{20}$ for equiv. conc. in benzene = −91·5°.	$[\alpha]_D^{20}$ for equiv. conc. in benzene = −92°.	$[\alpha]_D^{20}$ for equiv. conc. in benzene = −91°.

Equivalents of potash added. Resin calculated as having mol. wt. = 394.

	1			2			1		
Time min.	$[\alpha]_D^{20}$ °	$[\alpha]_{t_1} - [\alpha]_{t_2=230}$ °	k	$[\alpha]_D^{20}$ °	$[\alpha]_{t_1} - [\alpha]_{t_2=145}$ °	k	$[\alpha]_D^{20}$ °	$[\alpha]_{t_1} - [\alpha]_{t_2=215}$ °	k
2	+111·4	72·8	—	119·6	88·9	—	107·8	97·2	—
5	109·6	71·0	0·008	103·9	73·2	0·065	94·1	83·5	0·051
10	102·2	63·6	0·017	85·0	54·3	0·062	73·6	63·0	0·054
15	94·3	55·7	0·021	71·6	40·9	0·060	60·6	50·0	0·051
20	86·9	48·3	0·023	63·0	32·3	0·057	48·4	37·8	0·052
25	81·4	42·8	0·023	55·9	25·2	0·055	40·2	29·6	0·052
30	75·6	37·0	0·024	—	—	—	33·5	22·9	0·052
35	71·0	32·4	0·025	—	—	—	29·1	18·5	0·050
40	—	—	—	43·3	12·6	0·052	—	—	—
50	60·6	22·0	0·025	—	—	—	20·3	9·7	0·048
55	—	—	—	37·0	6·3	0·050	—	—	—
65	53·2	14·6	0·026	—	—	—	15·7	5·1	0·047
80	50·2	11·6	0·024	—	—	—	—	—	—
85	—	—	—	33·0	2·3	0·044	—	—	—
95	—	—	—	—	—	—	13·0	2·4	0·040
110	44·7	6·1	0·023	—	—	—	—	—	—
145	—	—	—	30·7	0	—	—	—	—
155	—	—	—	—	—	—	11·0	0·4	0·036
170	40·4	1·8	0·022	—	—	—	—	—	—
215	—	—	—	—	—	—	10·6	0	—
230	38·6	0	—	—	—	—	—	—	—
	Mean k (15–170 min.) = 0·0235 Mean k/c.c. methyl alcohol = 0·0117			Mean k = 0·0555 Mean k/c.c. methyl alcohol = 0·0139			Mean k (5–65 min.) = 0·051 Mean k/c.c. methyl alcohol = 0·0127		

From this table it can be readily seen that the resin of *D. malaccensis* reacts to caustic potash and methyl alcohol in a very similar way to the "Sumatra-type" resin. There is the same reduction in the rotation on adding methyl alcohol and the same conversion from laevo- to dextro-rotation on the addition of caustic potash in methyl alcohol to its benzene solution, and a subsequent decline in dextro-rotatory power with time. It would appear too as if increasing the methyl alcohol accelerated the decline. The magnitude of the initial change-over from laevo- to dextro-rotation is proportionately less than in the "Sumatra-type".

The results of adding caustic potash in methyl alcohol to the resin of *D. elliptica* and to rotenone in solution in benzene are given in Table IV. It will be observed here that although, as would be expected, the addition of methyl alcohol reduces the specific rotation, the addition of caustic potash in methyl alcohol does not produce the conversion to dextro-rotation, although it causes an immediate drop in the laevo-rotatory power, any subsequent change being probably not outside experimental error.

Table IV

The effect of adding caustic potash in methyl alcohol to benzene solutions of the resin of D. elliptica, *and of rotenone*

Description	Resin from *D. elliptica* Dilution			Rotenone	
	E.D.	E.D.	E.C.	*	†
Dilution in benzene g./100 c.c.	2·089	2·089	4·178	0·960	0·432
=g. root/100 c.c.	12	12	24	—	—
α_D^{20}	− 2·05°	− 2·05°	− 4·01°	− 2·19°	− 1·01°
$[\alpha]_D^{20}$	−98·4°	−98·4°	−96°	−228°	−234°
Methyl alcohol added to 5 c.c. of above	2 c.c.	4 c.c.	4 c.c.	4 c.c.	4 c.c.
Conc. after addition of methyl alcohol g./100 c.c.	1·492	1·161	2·321	0·533	0·240
$[\alpha]_D^{20}$ after adding methyl alcohol	−85·1°	−80·1°	−80·1°	−214°	−215°
$[\alpha]_D^{20}$ for equivalent conc. in benzene from graph	−99°	−99·5°	−98·5°	−232°	−233°
G.-equivalents of caustic potash added	1	2	1	2·1	5·3
$[\alpha]_D^{20}$ 5 min. after mixing	−9·38°	−0·86°	−7·32°	−79°	−79°
$[\alpha]_D^{20}$ after time in brackets	−0·67° (105 min.)	−2·58° (100 min.)	−8·61° (55 min.)	−68° (2731 min.)	−71° (1112 min.)

* Rotenone of equivalent strength to that present in *D. elliptica* resin E.C. Methyl alcohol and caustic potash as in E.C.

† Rotenone of equivalent strength to that present in *D. malaccensis* resin M.C. Methyl alcohol and caustic potash as in M.C.

Many other concentrations of rotenone in benzene ranging from 5 g. to 1 g. per 100 c.c. were tested with various amounts of methyl alcohol and caustic potash. The addition of methyl alcohol reduced the rotation slightly and caustic potash very materially and there was in the latter case a slight further loss with time, but in none did the rotation become positive. There is a tendency on the addition of caustic potash for crystalline matter to be precipitated if methyl alcohol is not present in considerable amount.

It is clear from these figures that the "Sumatra-type" and *D. malaccensis* resins contain ingredients that are not present to any extent in the sample of *D. elliptica* used. Rotenone, from the data given in Table IV, obviously plays no part in this characteristic switch-over in the rotation, and it is highly probable, from the results obtained with *D. elliptica*, that deguelin has no part in it either. Since the resins of the "Sumatra-type" and of *D. malaccensis* are distinguished from that of the *D. elliptica* by the large proportion of material extracted from their ether extracts by caustic potash, the fractions obtained in this way were tested. They were dissolved in benzene and caustic potash in methyl alcohol was added, the amount of caustic potash added being based upon an equivalence to toxicarol. The results are given in Table V.

Table V

Effect of addition of caustic potash in methyl alcohol upon benzene solutions of fractions from "Sumatra-type" resin

Material extracted and precipitated by caustic potash.
Conc. of solution = 3·234 g./100 c.c.
$\alpha_D^{20} = -1{\cdot}63°$. $[\alpha]_D^{20} = -50{\cdot}4°$.

2 c.c. methyl alcohol added to 5 c.c.
Conc. after addition of methyl alcohol = 2·374 g./100 c.c.
$[\alpha]_D^{20}$ after addition of methyl alcohol = $-22{\cdot}8°$.

4 c.c. methyl alcohol added to 5 c.c.
Conc. after addition of methyl alcohol = 1·797 g./100 c.c.
$[\alpha]_D^{20}$ after addition of methyl alcohol = $-6{\cdot}1°$.

Equivalents of potash added. Resin calculated as having mol. wt. = 410

1 | 1

Material soluble in ether after extraction by potash.
Conc. of solution = 3·018 g./100 c.c.
$\alpha_D^{20} = -2{\cdot}79°$. $[\alpha]_D^{20} = -92{\cdot}4°$.
4 c.c. methyl alcohol added to 5 c.c.
Conc. after addition of methy alcohol = 1·677 g./100 c.c.
$[\alpha]_D^{20}$ after addition of methyl alcohol = $-75°$.
Equivalent of potash added assuming mol. wt. = 394
1
Solution becomes very dark after addition of potash.
$[\alpha]_D^{20}$ = approximately $-12°$.
Little or no change in readings after 30 min.

Time min.	$[\alpha]_D^{20}$ °	$[\alpha]_{t_1} - [\alpha]_{t=210}$ °	k	$[\alpha]_D^{20}$ °	$[\alpha]_{t_1} - [\alpha]_{t=225}$ °	k
1	+266	206	—	+255	195	—
2	251	191	0·075	240	180	0·080
5	237	177	0·038	212	152	0·062
10	213	157	0·030	178	118	0·056
15	196	136	0·030	153	93	0·053
20	181	121	0·028	131	71	0·053
25	169	109	0·0265	115	55	0·053
30	157	97	0·026	104	44	0·051
35	145	85	0·026	92	32	0·053
40	137	77	0·025	86	26	0·052
45	128	68	0·025	80	20	0·052
50	120	60	0·025	—	—	
55	115	55	0·024	—	—	
60	111	51	0·024	70	10	0·050
75	96	36	0·024	65	5	0·050
90	85	25	0·024	—	—	—
105	78	18	0·023	62	2	0·044
120	72	12	0·024	—	—	—
150	65	5	0·025	—	—	—
165	—	—	—	61	1	0·032
180	62	2	0·026	—	—	—
210	60	0	—	—	—	—
225	—	—	—	60	0	—

Mean k (t = 10–90 min.) = 0·026 | Mean k (t = 10–75 min.) = 0·053

Mean k/c.c. methyl alcohol = 0·013 | Mean k/c.c. methyl alcohol = 0·013

The resin recovered from the material extracted and precipitated by caustic potash has been shown in Part II (5) of this series (p. 894) to be laevo-rotatory when dissolved in benzene. Its benzene solution reacts to the addition of caustic potash in methyl alcohol in a similar way to the resin of the "Sumatra-type" and of *D. malaccensis*. An instantaneous change-over to dextro-rotation takes place followed by an approximately unimolecular decline of the specific rotation with time. The magnitude of the initial change is, however, greater than it is with the crude resins.

The material left in the ether layer after extraction with caustic potash reacts more like *D. elliptica* and rotenone, in that there is a reduction in the laevo-rotatory power but no change of sign.

The separation of the fraction extracted and precipitated by caustic potash enabled us to test further the effect of methyl alcohol in accelerating the decline in the rotation after the addition of caustic potash. It will be seen from Table V and from Fig. 3, section II, that when the proportion of alkali to this resin is kept constant but the amount of methyl alcohol added to its solution increased twofold the rate of decline is practically doubled.

A repetition of the experiment with the potash extracted compound confirmed our view that the accelerating effect of methyl alcohol depended more on the proportion of the two solvents than on that of methyl alcohol to the resin. Three quantities of the "toxicarol" resin were weighed out and dissolved in benzene. The concentrations in the mixtures used are set out together with the mean velocity coefficients in Table VI.

Table VI

	Conc. in benzene meth. alcohol g./100 c.c.	Benzene c.c.	Methyl alcohol c.c.	KOH g. equiv.	Mean k (10–60 min.)	Mean k (whole of reaction)
(1)	2·286	5	2	1	0·022	0·020
(2)	2·286	5	4	1	0·048	0·047
(3)	1·143	5	2	1	0·025*	0·026

* This value becomes 0·022 if the final rotation at which the action comes to a standstill is taken as equal to that for Nos. 1 and 2.

If the effect depended on proportionality of methyl alcohol to resin we should expect the mean velocity coefficient of No. 3 to be on the higher level of No. 2, but if on that of the two solvents on the lower one of No. 1. The mean value for No. 3 is on the lower level.

It should, however, be pointed out that during the first 5 min. the reaction in the case of No. 3 was more rapid than in No. 1 and approximated to that of experiment No. 2, but its rate rapidly fell and for the next 50 min. was nearly the same as No. 1. The further decline in the rate of reaction was steady, and finally the velocity coefficient became less than 0·02.

In order to make it easier to compare the results tabulated in

Tables II–V, we have graphed the data in the sections of the Figs. 2, 3 and 4. In Figs. 2 and 3 the specific rotations, and in Fig. 4 the logarithms of the specific rotations minus the specific rotation at which the reaction approaches a standstill, are plotted against time in minutes.

Fig. 2, section I, illustrates the effect of increasing the amounts of caustic potash upon the induced dextro-rotation of the "Sumatra-type" resin. There is little or no alteration in the rate of decline whether 1, 2 or 3 g.-equivalents of potash are used. Fig. 2, section II, illustrates the difference in the rate of the decline of the induced specific rotation of different initial concentrations of the "Sumatra-type" resin in benzene. This change in rate is due, however, to the different amounts of methyl alcohol added with the caustic potash in the two cases. Fig. 3, section I, gives curves illustrating the decline in the induced dextro-rotation of the resin of the "Sumatra-type" and *D. malaccensis* as contrasted with that of *D. elliptica*, which remains laevo-rotatory, after treatment with equivalent amounts of potash in the same quantities of methyl alcohol.

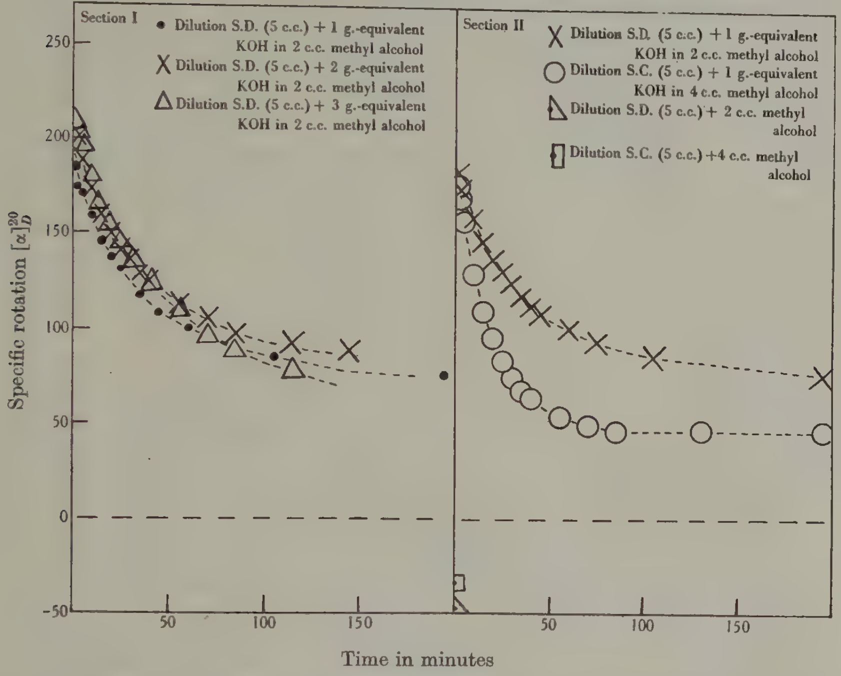

Fig. 2. Decline in the alkali-induced dextro-rotation of benzene solutions of the "Sumatra-type" resin. Section I, effect of concentration of potash. Section II, different concentrations of resin and methyl alcohol.

Fig. 3, section II, shows the effect of increasing the methyl alcohol upon the rate of the decline of the induced dextro-rotation of the benzene solution of the "toxicarol" resin. The velocity coefficient of the reaction is practically doubled by doubling the proportion of methyl alcohol to benzene solution of resin.

The semi-logarithmic graphs in the sections of Fig. 4 are intended to give a comparative conspectus of the relative rates of decline of the induced dextro-rotations on the addition of alkali in methyl alcohol to the benzene solutions of the resins of the "Sumatra-type" root, *D. malaccensis*, and the substance extracted and precipitated from their ether solutions by caustic potash ("toxicarol" resin). The effect of doubling the volume of methyl alcohol added with the caustic alkali is rendered evident by the steeper slope of the lines. That side reactions are taking place is evidenced by the fact that in several cases the points are only fitted by a straight line over a limited period. It is indeed rather surprising that the semi-logarithmic graphs approximate so closely to straight lines in so many instances, when the readiness with which some of the compounds, present in derris root, undergo chemical change in

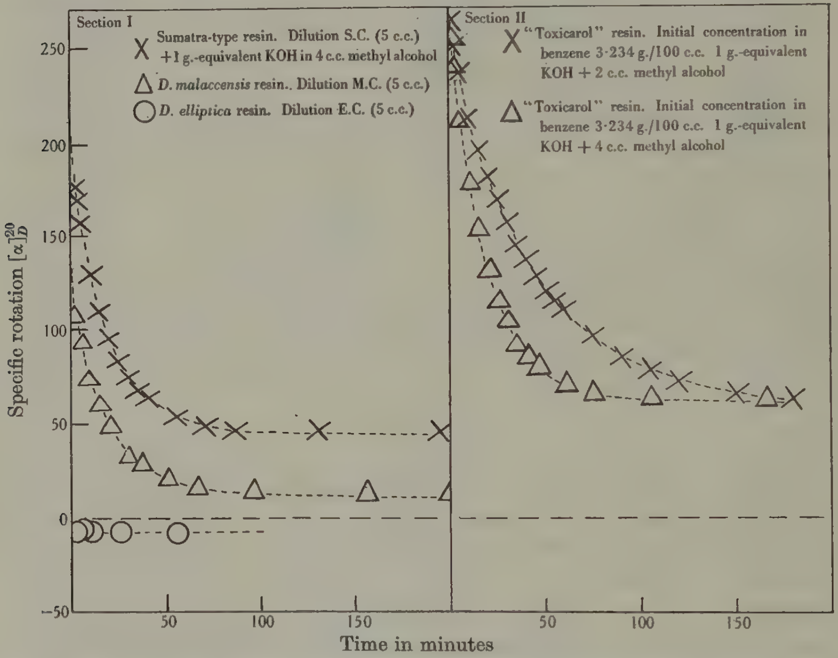

Fig. 3. Effect of adding caustic potash in methyl-alcohol upon the rotations of three types of derris resin and upon "toxicarol" resin (in solution in benzene).

the presence of alkali is considered. Indeed, most of the resins gradually darkened in colour after the addition of alkali until finally the benzene-methyl alcohol solutions were of such a deep red shade that accurate polarimeter readings were impossible. In addition, it is known that the specific rotations of the resins and of rotenone increase linearly with decline in concentration (Fig. 1); it is possible that a similar effect may

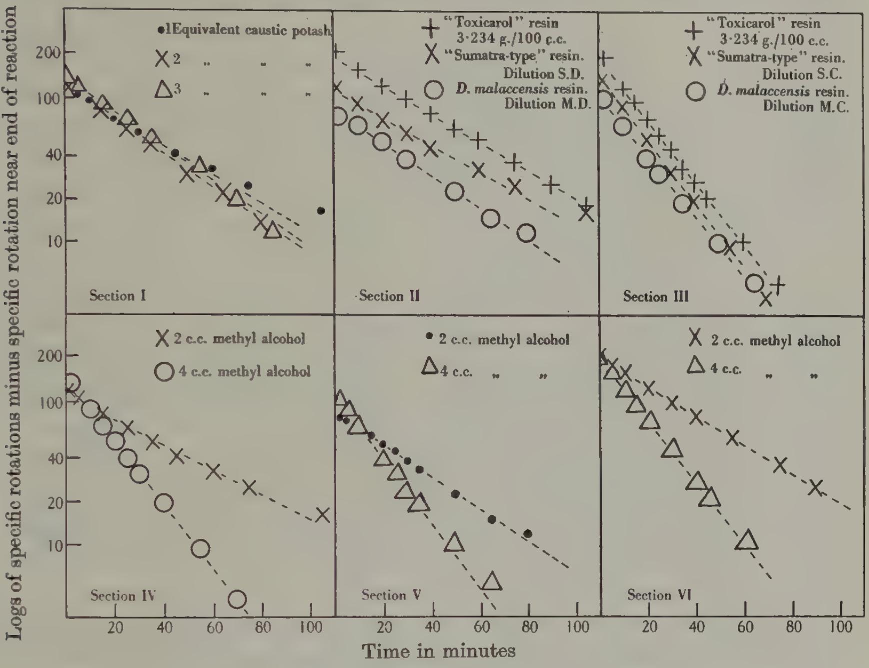

Fig. 4. Decline of induced dextro-rotation with time (semi-logarithmic scale).

Section I. "Sumatra-type" resin dilution S.D. in benzene with different amounts of caustic potash added in same quantity of methyl alcohol.

Section II. 5 c.c. benzene solutions of three resins + 1 g. equiv. caustic potash + 2 c.c. methyl alcohol.

Section III. 5 c.c. benzene solutions of three resins + 1 g. equiv. caustic potash + 4 c.c. methyl alcohol.

Section IV. 5 c.c. benzene solutions of "Sumatra-type" resin with 1 g. equiv. caustic potash and different amounts of methyl alcohol × dilution S.D. ○ dilution S.C. (p. 905).

Section V. 5 c.c. benzene solutions of *D. malaccensis* resin with 1 g. equiv. of caustic potash and different amounts of methyl alcohol • dilution M.D. △ dilution M.C. (p. 907)

Section VI. 5 c.c. benzene solutions of toxicarol resin with 1 g. equiv. of caustic potash and different amounts of methyl alcohol. Initial concentrations in benzene were the same (3·234 g./100 c.c.). (p. 909).

characterize the dextro-rotatory compound, and thus the rotations observed during the reaction may not have exactly the same equivalence as a measure of the concentrations of the reacting substance.

CONCLUSIONS

Owing to the large variations in type characterizing derris root, as many chemical and insecticidal data as possible are required about its different varieties. It seems to us that only in this way can the existing confusion be cleared away, or accurate information be made available as to the effect of genetical, soil, or climatic factors upon the quality of these plants. It is possible that in the course of time only a few varieties or types will be produced under cultivation and thus the problem of the assessment of their value be simplified; but at present there is little assurance that consignments as they come on to the market can be put into any one category as far as the chemical characteristics, botanical make-up and insecticidal potency are concerned. The evaluation by chemical means is one of great difficulty and is rendered more so by this contingency. The fractionation therefore of the resins of derris and cubé and the determination of the chemical characteristics and insecticidal potency of the fractions appears to be a matter of some urgency if the methods of assessment are to be improved.

In the foregoing section a study has been made of the optical activities of the benzene solutions of resins and their fractions derived from three samples of derris root containing approximately the same amounts of ether extract. They were *D. elliptica*, *D. malaccensis*, and the "Sumatra-type" of Cahn & Boam. They were all laevo-rotatory, the specific rotations being in descending order of magnitude *D. elliptica*, *D. malaccensis*, the "Sumatra-type". The rotations did not give a quantitative measure of the relative toxicities of the roots to *Aphis rumicis*, nor was it possible to obtain a quantitative assessment of the relative toxicities by an examination of the rotations of their fractions.

The addition of methyl alcohol to solutions of rotenone and of the resins reduced their specific rotations. A preliminary attempt to fractionate the resin of the "Sumatra-type" by means of methyl alcohol gave a fraction which was dextro-rotatory in solution in methyl alcohol but laevo-rotatory in benzene. The resins of the "Sumatra-type" and of *D. malaccensis* when dissolved in benzene and treated with a standard solution of caustic potash underwent a change of sign in their optical rotation. There followed a decline in the rotation on standing, the time reaction being approximately unimolecular in type although side reactions apparently accompanied it. The speed of the decline was only slightly affected by the amount of caustic potash added or by the amount of resin present, provided the amount of methyl alcohol added to the same volume of solution was the same. An increase in the amount of methyl alcohol added with the caustic potash accelerated the decline in activity. In our experiments the rotations never reached zero after the initial change of sign. Rotenone and the resin of *D. elliptica* tested did not undergo a change of sign under these conditions, but the normal laevo-rotation was reduced instantaneously and then either slowly declined or remained approximately constant. The "Sumatra-type" root was therefore much more like *D. malaccensis* than *D. elliptica.*

When the ether solutions of the "Sumatra" or *D. malaccensis* resins were treated with caustic potash, a portion was extracted and precipitated by the alkali and a portion remained in solution in the ether (see Part II of this series, p. 887)(5). The yellow precipitate, on acidification and extraction, gave a resinous product[1] which was laevo-rotatory in benzene solution but underwent the instantaneous change of sign when caustic potash was added in methyl alcohol. The rotation decreased on standing and the velocity of this reaction was also accelerated by an increase in the amount of methyl alcohol added with the caustic potash. The fraction soluble in ether behaved like the resin extracted from *D. elliptica*, showing no change of sign of rotation on the addition of caustic potash in methyl alcohol to a benzene solution. It appears probable that the material extracted from ether solutions of the "Sumatra-type" or *D. malaccensis* contains the precursor of toxicarol in high concentration.

Further work on these lines is required in order to ascertain the validity of the reaction as a diagnostic sign and to discover whether rotation methods would be of use in ascertaining the amount of "toxicarol" present in a sample of roots and if its presence is determined by genetical, climatic or cultural conditions.

SUMMARY

A study has been made of the rotations of the resins from three types of derris root, and of a fraction rich in toxicarol separated from two of them. No strictly quantitative relationship between their rotations and their toxicities to *Aphis rumicis* has been found. The addition of caustic

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[1] Since this paper was sent to press a crystalline derivative, showing the characteristic change of sign in rotation on the addition of caustic potash in methyl alcohol to its benzene solution, has been isolated from this resin.

potash in methyl alcohol to the benzene solutions of the resins induces a characteristic change from laevo- to dextro-rotation in the samples rich in toxicarol. The induced dextro-rotation then declines in value with time. This effect is shown by the "toxicarol" resin. The rate of the decline is accelerated by increasing the amount of methyl alcohol.

We are greatly indebted to the Copper Technical Bureau for gifts of the "Sumatra-type" root and of resins derived from this strain.

We wish to express our thanks to Mr C. Read for help during the course of this work.

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REVIEWS

Tobacco Diseases and Decays. By F. A. Wolf. Pp. xix + 454, with a frontispiece and 111 figs. Duke University Press. 1935. $5.00.

Twenty years ago the literature of tobacco diseases was comparatively slight but, during the two decades, the attention given to the subject has been so great that the bibliography in the present volume runs to over 1000 citations. The time was obviously ripe for a critical survey and synthesis of this mass of publication and it was fitting that this should be done by Professor Wolf who, during this period, has contributed materially to our understanding of the problems. As tobacco is cultivated intensively in many parts of the North Temperate, Torrid, and South Temperate Zones the literature is written in many languages and is scattered through numerous journals. A comprehensive survey and critical ordering of the data can have been no easy task and the successful outcome of the author's labours has placed plant pathologists in his debt. The book is well written although, here and there, it contains some awkward sentences and it will be useful to growers, agricultural officers and investigators alike, since it not only gives a clear picture of the present state of our knowledge but points out clearly the gaps and makes suggestions for further research.

The author has not followed a uniform plan of discussion of each disease but, in general, has described the symptoms of the disease, then considered the cause of it and the conditions which favour its development and dissemination, and finally discussed palliative and remedial measures. References are appended to the consideration of each disease. As with so many crops, knowledge of the more academic aspects of tobacco diseases is greater than knowledge of their field control and, as the author says, "The urgent need for experimentation on control of many of the diseases (this work to be conducted in the field and not in the laboratory or the greenhouse) is all too apparent to anyone who attempts to aid growers in their tobacco disease problems."

Following an introductory chapter, there is a discussion of the seed bed as related to the problem of the control of tobacco diseases. Chapter III contains an excellent account of nutritional diseases, chapter IV of those due to unfavourable water relations, ending with the rather delightful sequence of "Wet Weather Spot", "Blackfire" and "Flop", and chapter V of disorders and diseased conditions that are little known and non-infectious, special attention being given to "Frenching". Chapter VI commences the portion devoted to parasitic diseases and is an admirable presentation of our knowledge of those due to viruses. It is not likely that all pathologists will agree with the author's opinion, p. 112, that "It would appear entirely probable that some viruses may be non-living, others living", but it is an interesting idea. Also, the author's suggestions on p. 116 for a distinctive virus terminology, e.g. "Virusation, virusization, or enphytization, instead of inoculation" will not meet with unqualified approval. Chapter VII contains an account of bacterial diseases and chapter VIII of diseases of the growing crop caused by fungi. There is little to be said about these chapters: they are just good straightforward accounts of the diseases concerned. Two short chapters follow treating, respectively, of diseases caused by nematodes and of those caused by parasitic flowering plants. The final chapter contains a useful account of the decays of tobacco during curing, fermentation and storage and after manufacture, and the book closes with a splendid bibliography and a good index.

The work is illustrated by 111 text-figures which, with few exceptions are photographs of disease symptoms in leaves and plants. Some of these are original and most of them are good but Figs. 81, 84, 92 and 95 are not up to standard. The author writes essentially as a plant pathologist and not as a mycologist or bacteriologist. In consequence the mycological and bacteriological details of the parasites receive comparatively little attention, only one fungus, for example, being illustrated in the text-figures. The book is well printed and produced and is a valuable addition to the literature of plant pathology.

WILLIAM B. BRIERLEY.

Diseases of Vegetable Crops. By J. C. Walker. Pp. 65. The Author, Horticultural Building, Madison, Wisconsin, U.S.A. 1935. $1.60.

This book is an extremely condensed summary of the fungal, bacterial, virus and nematode diseases of asparagus, onion, crucifers, bean, pea, cucurbits, tomato, potato, pepper, egg plant, celery, carrot, beet, spinach, parsnip, rhubarb, okra, lettuce, and sweet potato. Brief notes are given on the culture of each crop plant and each major disease is treated under the following heads: hosts, history, occurrence, economic importance, symptoms, causal organism, life history of causal organism, pathological histology, environal relations, varietal resistance, and control. Minor diseases are listed. To each disease is appended a number of key references and the book closes with an index of diseases arranged alphabetically under hosts.

Considering the number of diseases included and the small space available for each, the book contains an astonishing amount of information. Not only is it a first class summary of the major diseases of vegetable crops but, for students of plant pathology, it will serve as an admirable guide to this portion of the subject. The text is a photolithoprint of the author's typescript including misprints and, being reproduced on one side of the page only, leaves a useful blank side for personal annotations.

WILLIAM B. BRIERLEY.

Bacterial Plant Diseases. By A. Jaczewski. Posthumous work revised and enlarged by N. Naumov. Pp. viii+709, 120 figs. Moscow: State Printing Office. 1935.

With the exception of English translations of Title Page and Contents this large volume is entirely in Russian. The chapters run as follows: I, Development of knowledge of bacterial plant diseases; II, Morphology and physiology of bacteria; III, Conditions affecting bacterial activities; IV, Multiplication of bacteria; V, Life cycles of bacteria and classification; VI, Role of bacteria in nature; VII, The mutual interactions of bacteria and their symbiotic relations; VIII, The mode of infection of the host and its response to attack; IX, Geographical distribution of bacteria and the mode of their dissemination; X, Methods of investigation of bacterial plant diseases. Nutrient media; XI, Methods of control; XII, Bacterial plant diseases systematically arranged under bacterioses of A, Cryptogams; B, Conifers; C, Monocotyledons; D, Dicotyledons. There are appendices containing a table of morphological and biochemical properties of plant pathogenic bacteria, miscellaneous remarks about staining and a bacterial descriptive chart. The bibliographical index of 1734 citations is arranged as follows: A, General author index; 1, Russian and Bulgarian papers; 2, Foreign papers; B, systematically arranged index on main subjects. The volume closes with subject, host and pathogen indexes in Roman characters. In the first nine chapters containing the general part of the work references are also given as footnotes to the text. The illustrations are all text-figures and, owing to the coarse quality of the paper, are often unsatisfactory.

It is to be hoped that arrangements will be made for an English translation of this volume which, so far as one can judge, is a work of considerable scientific importance. Such a publication would be a fitting memorial to its distinguished author.

WILLIAM B. BRIERLEY.

The Cultivated Races of Sorghum. By J. D. Snowden. Pp. vii+274, 3 plates and 31 text-figs. London: Adlard and Son. 1936. 10*s*. 6*d*.

Of *Sorghum vulgare* Hitchcock recently stated "The differences between most of the varieties are so indistinct and so unstable because of intercrossing as to make it very difficult to assign descriptive limits. The application of botanical names is uncertain, and it seems best, therefore, not to assign to them definite varietal or specific Latin names." Mr Snowden has taken his courage in both hands and in the present work has described thirty-one species with numerous varieties, races and forms.

The first chapter is an admirable discussion of the botanical history of the cultivated Sorghums and presents sound justification of the author's recognition of the more distinct types as definite units with specific status. In the second chapter, on classification, the author separates off *Sorghastrum* leaving a more uniform genus *Sorghum* which he divides into two well-marked sections, *Eu-Sorghum* and *Para-Sorghum*. The former is then divided into two subsections, *Arundinacea* and *Halepensia*, and subsection *Arundinacea* into two series, *Spontanea* and *Sativa*. The latter contains all the species cultivated for their grain or sweet stems and is classified into six subseries which are described botanically. There follow brief discussions of the general distribution of *Sorghum* and of the significance of the botanical characters. A key to the subseries and species leads to the main part of the book which is a detailed description and enumeration of the species, varieties and forms and includes not only taxonomic matter but also useful notes on the history, distribution, culture and economic importance of the plants. The skilful use of spacing and different types renders this part of the work very clear. The final chapter is an interesting consideration of the relationships of the wild and cultivated Sorghums, and the book closes with a bibliography of over 400 references, arranged by dates under subjects, and indexes of botanical names (including pre-Linnean nomenclature), vernacular names, and countries and regions. The book is illustrated by three plates and thirty-one excellent line drawings.

Although the work was primarily done by Mr Snowden, it was carried out at Kew with the active co-operation of the officers of the Agricultural Departments of the British Empire, and it sets a valuable precedent which, it is to be hoped, Kew will be able to follow with other crop plants. Botanists and agriculturists will accord thanks not only to the author for his painstaking labours but to the Trustees of the Bentham-Moxon Fund who generously subsidized the work and published the results in so admirable a form.

WILLIAM B. BRIERLEY.

The Algae and their Life Relations: Fundamentals of Phycology. By J. E. Tilden. Pp. xii + 550, frontispiece and 257 figs. Minneapolis: University of Minnesota Press. 1935. 22*s*. 6*d*.

The Algae have always proved a fascinating study to botanists and students have been fortunate in having available the great works of Oltmanns and West. There have now been published, almost simultaneously, two comprehensive treatises by life-long students of this group, Fritsch and Tilden, although of the former book, volume one only has yet appeared. In fact, the Algae, the direct economic importance of which is comparatively slight, are, in many respects, better known than other groups of plants of vital importance to mankind. It is a strange kink in the academic mind which puts economically unimportant things first and neglects things of equal scientific interest and transcending human value.

In chapter I the author discusses various hypotheses concerning the phylogeny of the Algae, emphasizing the theory of parallel development. A consideration of the distribution of marine Algae in time and space in which the author endeavours to show that this has been determined by solar energy, is followed by an interesting chapter on algal classification based on evolutionary development, with special reference to pigmentation and food reserves. The next 424 pages contain the classificatory and descriptive portion of the book, the author working steadily through the Cyanophyceae, Rhodophyceae, Phaeophyceae, Chrysophyceae and Chlorophyceae. Chapter IX contains a very brief discussion of algae control in polluted waters, and the following two chapters a discussion of Algae as food for animals and man. The latter chapter is distinctly interesting and unexpected in a book of this nature, although it is doubtful whether the unique claim made for the Algae can be substantiated. There is a valuable appendix on the standardization of methods of drawing Algae for publication which all botanists will find useful, and the book closes with a selected bibliography and a good index.

The book is primarily a text for advanced students in botany and is written in clear and simple language, technical terms having been simplified and reduced in number. The beautiful illustrations, many of which are original, are a valuable and striking feature, and good use is made of extensive tables and diagrams. The book is finely printed and produced and is a notable contribution to algal literature.

WILLIAM B. BRIERLEY.

Protoplasm. By W. SEIFRIZ. Pp. x+584, 179 figs. London: McGraw-Hill Book Co. 1936. 36*s*.

During the last twenty years Prof. Seifriz of the University of Pennsylvania has studied the structure of the living cell, using especially micrurgical technique, and a noteworthy part of our present knowledge derives from his pioneering investigations. In spite of the issue of such journals as *Cytologia* and *Protoplasma*, the literature dealing with these problems is very widely scattered, the author's own contributions appearing in over sixteen journals. This is due to the fact that protoplasm is a focus of many sciences and research on the problems of each particular aspect is published in the journals of the special science.

In the present volume the author has endeavoured to bring together "all those parts of the branches of science which bear upon the physical chemistry of living matter" and "to indicate how far research has gone in the application of physics and chemistry to those biological phenomena which can be reduced to cellular or protoplasmic processes". Most of these researches are extremely technical and rendered almost unintelligible to many biologists by presentation in a maze of mathematical or statistical symbolism. The author has not, of course, been able to avoid formulas, curves and tables but has reduced them as far as is consistent with accuracy and completeness since, as he says, "a situation which can be clearly put into words becomes meaningless to the non-mathematical mind when stated in terms of formulas and curves." This, alone, would make his book welcome to biologists.

Chapters I–V are introductory, the first two dealing with the living substance and the cell, and the following three with methodology. The next sixteen chapters contain a masterly survey and discussion of the physical chemistry of protoplasm, and the four which follow, of its chemical constituents. The last two chapters deal respectively with regulatory substances and the problem of the origin of living matter—this last a highly controversial chapter! The book closes with a useful selected bibliography of about 550 citations arranged by subject, and a good index.

For his consideration the author has selected the more important lines of research but the book, although fairly comprehensive, makes no pretence of encyclopaedic treatment. He presents fairly both sides of any question in discussion but states clearly his own views, believing that "a definite but not too arbitrary stand is more conducive to a clear grasp of a subject than a wholly impartial point of view" and that "a book wholly devoid of contention is likely to be less stimulating than one which occasionally indulges in healthy disagreement". In spite of the clear and persuasive writing of Prof. Seifriz, and his cogent argument, probably many readers will, here and there in the book, find themselves pulled up by statements and viewpoints affording scope for most healthy disagreement. As the author says, however, "change and doubt are the very spirit of scientific thought", and he himself is so careful to avoid finality in statement and severely adverse or merely destructive criticism that the interest and fairness of his presentation almost disarm opposition.

Owing to the scattered literature, the number and wide range of the data and the abstruse technical character of so many of the investigations, most biologists are unable to keep a general and up-to-date viewpoint in relation to these problems, or feel themselves quite incompetent to evaluate the evidence or see its trends and implications. In surveying this work and marshalling the data in the light of his special knowlege and long and intimate experience of actual research on a wide range of protoplasmic problems, the author has performed a valuable task and, in writing his book in so interesting, clear and stimulating a manner, he has added to our debt.

The book is a notable achievement and it will be of interest and value to all biologists since, whatever their special fields of research, all their problems originate in the structure and behaviour of protoplasm. WILLIAM B. BRIERLEY.

Evolution. By A. FRANKLIN SHULL. Pp. x+312, 64 figs. London: McGraw-Hill Publishing Co., Ltd. 1936. 18*s*.

Even the most applied of biologists never loses his interest in fundamental problems of evolution and, in view of the evolutionary developments of statistics and genetics during the last decade, is wise if he keeps in close touch with this general field. Few books on evolution, however, give one any idea of the present day concept of that process and, particularly, lack any adequate application of genetical and statistical knowledge to the problems of evolution. What has been needed is a book which, whilst general in scope, attempts to show precisely how evolution works in the presence of the typical heredity mechanism, i.e. which reviews the field of evolution as it appears to modern biologists, with the genetic bearings indicated wherever these may reasonably be assumed. The present book meets this need admirably and is an excellent piece of work.

The first four chapters are introductory and present a clear picture of the general lines of evidence for evolution. The next six chapters contain a suggestive discussion of the relation of the material basis of evolution to the facts of heredity, and the following three chapters a critical analysis of the present status of natural selection. Three chapters are then devoted to a consideration of adaptive and non-adaptive characters, and the role of genetic and environmental isolation in the formation of races and species. These are followed by an interesting but somewhat woolly chapter on the evolution of evolution, and a final chapter which considers destructively the idea of emergent evolution. The book contains a bibliography which, although selected contains some rather striking omissions, in particular reference to important Russian work, and closes with an index.

The volume is written largely from a zoological standpoint, although it attempts to be general in its scope, and is in such clear language that difficult and obscure problems appear almost too simple. It is an interesting and suggestive book and one to be recommended. WILLIAM B. BRIERLEY.

Sea Trout and Trout. By W. J. M. MENZIES. Pp. 230, 17 plates and 3 text-figs. London: Edward Arnold and Co. 1936. 10*s*. 6*d*.

Mr Menzies, who is Inspector of Salmon Fisheries of Scotland, is well known as an authority on the salmon and the rather delightful little book which he has now written on the trout will add to his reputation among anglers and naturalists. After describing the distribution and development of the migratory and non-migratory races of trout, and the life history of the sea trout in some detail, the author gives an interesting account of the ecological relationships of trout and outlines the methods of observation, marking of fish and scale study which have led to our present knowledge. There is a somewhat slight chapter on diseases, abnormalities and parasites and a final chapter in which the author indicates ways in which research may be further applied in the preservation and improvement of fisheries. The book opens with an appreciative foreword by G. Herbert Hall, himself a well-known authority on salmon and trout, and closes with a short bibliography and an index.

The book is well illustrated and produced and, although the writing is interesting and popular, it is, as one would expect from its author, accurate scientifically. The book should have a wide appeal. WILLIAM B. BRIERLEY.

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